100 Years Since Tolaas: Bacterial Blotch of Mushrooms in the 21st Century

Ebrahim Osdaghi¹, Samuel J. Martins², Laura Ramos-Sepulveda³, Fabrício Rocha Vieira², John A. Pecchia², David Meigs Beyer², Terrence H. Bell², Yinong Yang², Kevin L. Hockett^{2,†} and Carolee T. Bull^{2,†}

¹ Department of Plant Protection, College of Agriculture, Shiraz University, Shiraz 71441-65186, Iran

² Plant Pathology & Environmental Microbiology Department, The Pennsylvania State University, University Park, PA 16802, U.S.A.

³ Department of Biology, Millersville University of Pennsylvania, Millersville, PA 17551, U.S.A.

Keywords: *Agaricus bisporus*, phage therapy, *Pseudomonas* spp., tolaasin, virulence factors, white line-inducing principle, microbiome, devome, compost, casing

Agaricus bisporus is one of the most widely cultivated edible mushrooms around the globe. According to FAOSTAT (<http://www.fao.org/faostat>), the worldwide net production value of mushrooms has reached over US \$19.46 billion in 2016 (FAOSTAT 2017). Mushroom production in the U.S. has increased from 70.3 thousand tonnes (t) in 1967 to 415.9 t including 404 t of button mushroom (*A. bisporus*) and 11.7 t of specialty mushrooms (e.g. *Pleurotus* spp., *Letinula edodes*, etc.) in 2017 (USDA NASS 2018; Supplementary Fig. S1A). Furthermore, since about 2000, demand for organic mushrooms has increased in the U.S. While only 3.8 t of organic mushrooms were produced in 2000, the amount has increased to 58 t (almost 14% of total mushroom production) in 2017 (USDA NASS 2018). Button mushrooms accounted for 83% of the mushrooms sold as certified organic, while all specialty mushrooms made up the remainder. Among the 307 mushroom producers in the U.S., 80 growers (26% of total growers) produced certified organic mushrooms in 2017 (USDA NASS 2018). Indeed, the term “organic mushroom” refers to diverse production procedures and safety criteria in different countries (e.g., E.U. vs. U.S.A.). In this manuscript we are referring to organic mushroom production in the U.S. as defined by the U.S. National Organic Program. U.S. organic production is regulated: at site selection with regards to buffer zones, at composting, with regards to the synthetic substances allowed, and natural products not allowed. These standards can differ significantly from those of other countries or regulatory groups. For example, in the

U.S., raw materials for compost do not have to be from organic production because organic product is not available. In contrast, in the E.U., organic mushrooms must be grown on compost made from organic straw (grown without pesticides on land) and organic poultry manures. These differences may influence disease incidence, management, and the dynamics of the microbiome in mushroom production.

A number of bacterial (Fermor 1987), fungal (EPPO 2010), and viral (Eastwood et al. 2015) diseases were reported to reduce quality and quantity of mushroom products. Due to the limited options for use of conventional chemicals and pesticides in mushroom production, biotic constraints (e.g., fungal and bacterial pathogens) are considered the main bottlenecks in the mushroom industry, especially where pesticide-free production is desired. Among biotic constraints in mushroom production is the colonization of mushroom caps by pathogenic bacteria, which leads to cap discoloration and pin death. Although yield loss due to bacterial blotch has been attributed to *Pseudomonas tolaasii*, the causal agent of bacterial blotch (Paine 1919; Tolaas 1915), other *Pseudomonas* species induce similar diseases on edible mushrooms. *P. agarici* causing drippy gill or yellow blotch (Young 1970), *P. costantinii* causing brown blotch (Munsch et al. 2002a), and two taxonomically invalid species ‘*P. reactans*’ (Iacobellis and Lo Cantore 2003) and ‘*P. gingeri*’ (causing ginger blotch; Wong et al. 1982) are responsible for cap discoloration generally termed bacterial blotch (Godfrey et al. 2001; Sajben et al. 2011; Schisler et al. 1968). Here and elsewhere the term “blotch” refers to a group of diseases all caused by different species of pseudomonads. Although some differences in symptomology have been associated with different pathosystems, the correlations between a given pathogen/species and specific symptoms (e.g., yellow blotch and *P. agarici*) have not yet been rigorously elucidated since the methods used to identify pathogens were unsuitable until recently (see Taxonomy of the Causal Agent(s)) especially when they occur in mixtures. In this review, unless otherwise stated, the term blotch corresponds to the brown blotch disease caused by *P. tolaasii* or blotch of unknown etiology.

Bacterial blotch was described for the first time in 1915 (Tolaas 1915) and is still considered the most important mushroom disease in terms of economic loss and quality reduction (Soler-Rivas et al. 1999a). The disease significantly reduces the marketability of mushrooms due to the discoloration of the cap surface to a dark yellow or brown and a change in the texture. Although the button mushroom (*A. bisporus*) is considered to be the main host of

†Corresponding authors: C. T. Bull, ctb14@psu.edu; and K. L. Hockett, klh450@psu.edu

Funding: This study was funded by the USDA National Institute of Food and Agriculture and Federal Hatch Appropriations PEN04589, and PEN04648 and accession numbers 015871, 1006639, the Mushroom Research Competitive Grants Program - Giorgi Mushroom Company (CTB, LRS, and SJM), and through a grant (USDA-NIFA-OREI) #2017-02421 to DMB, JAP, KLH, and CTB.

* The e-Xtra logo stands for “electronic extra” and indicates that four supplementary figures and one supplementary table are published online.

The author(s) declare no conflict of interest.

Accepted for publication 13 August 2019.

P. tolaasii, the pathogen has also been reported to cause disease on other mushrooms such as *A. bitorquis*, *A. campestris*, *Flammulina velutipes*, *Lentinula edodes*, *Pleurotus eryngii*, and *Pleurotus ostreatus* (Fletcher 1979; Lee and Cha 1998; Rainey et al. 1992; Tsuneda et al. 1995).

Controlling relative humidity and temperature – to avoid excessive condensation of water on mushroom caps – and elimination of the pathogen by periodically cleaning mushroom facilities with disinfectants (e.g., chlorine) are the most effective methods for management of bacterial blotch disease (Fletcher et al. 1989). However, since the 1970s, many investigations have been carried out to develop additional sustainable and eco-friendly methods to be used in mushroom production. Regular application of chlorinated water onto the casing layer (see below), in integration with the control of environmental factors, as well as biological control using mushroom-associated microorganisms are considered the best strategies on conventional farms (Fletcher and Gaze 2007; Lomax 2007; Navarro et al. 2018; Savoie et al. 2016).

This review provides an up-to-date treatment of the factors related to bacterial blotch including disease etiology, placing the disease within a mushroom microbiome context, taxonomy, and pathogenicity mechanisms of the causal agent(s), as well as different management strategies proposed for use in organic

mushroom production. We also discuss the environmental conditions that help control the disease in production facilities. Furthermore, a number of promising biocontrol methods (e.g., antagonistic microorganisms and phage therapy) and gaps in their application are discussed in depth to provide a guideline to develop a biological control cocktail for bacterial blotch management.

Background

Symptomology. Symptoms of bacterial blotch mostly emerge on the cap surface of mature mushroom, diminishing fresh mushroom marketability. However, all the mushroom growing stages in production facilities, as well as post-harvest storage and sales, promote environmental conditions that allow bacterial blotch pathogens to proliferate. Figure 1 illustrates elements of the commercial mushroom production process and references how each phase of production interacts with the pathogen and bacterial blotch development within the production facilities. Initially, small, irregular, yellow spots appear on mushroom caps (Fig. 2A). Discoloration is pale yellow or yellowish brown to tan or light brown at the start (Fig. 2B) and darkens to a golden yellow or dark brown color (Fig. 2C, D). The degree of discoloration depends on timing of infection, environmental conditions, bacterial strains, as well as the density of bacterial populations on the caps (Cho and Kim 2003; Soler-Rivas et al. 1999a).

Fig. 1. Edible mushroom (*Agaricus bisporus*) production procedure in commercial farms, and the factors influencing incidence of bacterial blotch in each step. While entire elimination of blotch pathogen(s) in the composting and casing steps is almost non-achievable, management of relative humidity, temperature, and watering practices in pinning and harvesting steps would lead to a significant decrease in blotch incidence in the farm. Sanitary practices in harvesting will prevent the spread of blotch pathogen during the second and third breaks. Blotch incidence is also affected by mushroom cap microbiome in the cropping process. Although a number of pseudomonads (i.e., *Pseudomonas putida*) in casing are mandatory to promote cap development, several taxonomically diverse species (see section: Taxonomy of the Causal Agent) induce blotch symptoms. At the same time, the cap microbiome may include antagonistic strains that inhibit blotch disease under environmentally favorable conditions.

Darker discoloration is often seen under optimal environmental conditions when infection occurs early in development of the pins and can rarely lead to total death of the pins/buttons. The most severe symptoms are deep browning or pitting on the surface of the cap, which occur in the presence of free moisture and high relative humidity in the house and can cause entire malformation of the mushroom caps (Fletcher et al. 1989). See Supplementary Figures S2, S3, and S4 for the diversity of blotch symptoms.

Taxonomy of the causal agent(s). Bacterial blotch caused by *P. tolaasii* was first described by A. G. Tolaas (1915) while working at the University of Minnesota, U.S.A. Tolaas referred to the symptomatology previously described in France by Constantin in 1892 and Matruchot in 1909, but it was S. G. Paine who described the organism in 1919 and named it *P. tolaasii* to acknowledge the work of Tolaas (Paine 1919). To date, all pathogens attributed as causal agents of bacterial blotch have been identified as members of the ecologically and phenotypically diverse genus *Pseudomonas*. The genus was initially a repository for bacterial species that did not fit in other taxa, *Pseudo* from the Greek for false and *monad* from the Greek for unit, which comes together to mean “false unit” (Palleroni 1984, 1993). *Pseudomonas* spp. are nutritionally versatile and are isolated from a wide variety of environmental habitats, including mushrooms and the compost that they grow in (Moore et al. 2006; Samson et al. 1987; Schroth et al. 2006). There are two major groups of pseudomonads (Palleroni 1993), and bacterial pathogens of mushrooms have thus far all been identified as members of the *Pseudomonas fluorescens* group.

In the last 30 years, a number of other pathogens have been described that cause blotching symptoms, including *P. agarici* and *P. costantinii*. Additionally, two invalidly named species, ‘*P. gingeri*’ and ‘*P. reactans*,’ cause blotching or discoloration of the mushroom cap or deterioration of the cap and gills (Henkels et al.

2014; Munsch et al. 2002a; Ramette et al. 2011; Wells et al. 1996; Wong et al. 1982; Young 1970). Furthermore, recent work by Henkels et al. (2014) indicates that a much broader diversity of *Pseudomonas* species, including *P. protegens*, can cause mushroom cap discoloration, though in our work, we have never isolated a related pathogen from mushroom production houses (Martins, Ramos-Sepulveda et al., *unpublished data*). Godfrey et al. (2001) described *Pseudomonas* sp. NZ17 in New Zealand and the following year, Munsch et al. (2002a) published their work on *P. costantinii*. Other less common causal agents of blotch diseases of mushroom were described later, which were commonly confounded with *P. tolaasii* or *P. fluorescens* due to the lack of adequate methods for distinguishing among *Pseudomonas* species.

Related diseases like ginger blotch and drippy gill are also caused by *Pseudomonas* species. Though not a valid name, ‘*P. gingeri*’ was first described by Wong et al. (1982) in England. They described the blotch caused by this pathogen as ginger instead of brown. The first confirmed account of drippy gill was in 1970 in New Zealand, and the pathogen was identified as *P. agarici* (Young 1970). The disease has been slowly spreading to different parts of the world. For example, Iacobellis and Lo Cantore (2003) described drippy gill for the first time in Italy in 2003.

Until recently, methods for classification and identification of species within the genus *Pseudomonas* were unsatisfactory (Bull and Koike 2015). Lelliott et al. (1966) devised a scheme to help differentiate seven groups of pseudomonads using five determinative tests (LOPAT - levan production, oxidase, potato soft rot, arginine dihydrolase, and hypersensitive reaction in tobacco leaves). As can be seen from Table 1, the LOPAT tests do not distinguish among the organisms that cause bacterial blotch. Wong and Preece (1979) introduced the “white line” test as another determinative assay useful for identifying *P. tolaasii*. *P. tolaasii* was thought to be easily identified

Fig. 2. Symptoms of bacterial blotch symptoms on mushroom caps. Golden yellow (A, B) or dark brown (C, D) discoloration reduce the marketability of the products.

by a specific interaction between the pathogen and the organism by the invalid name '*P. reactans*,' which is commonly found in the casing layer, compost, and mushroom caps (Soler-Rivas et al. 1999a; Wong and Preece 1979). When these species are streaked side by side on King's Medium B (KB), a white precipitate can be seen between the two cultures (Fig. 3). The white line is the result of an interaction between tolaasin produced by *P. tolaasii* and a substance white line inducing principle (WLIP) produced by '*P. reactans*' (Rainey et al. 1992; Wong and Preece 1979). A Tn5-generated mutant of *P. tolaasii* (NCPBP 1116), which was tolaasin-negative and non-pathogenic on mushroom caps, did not produce a white line with the standard '*P. reactans*' strains (Rainey et al. 1993), while the type strain of *P. tolaasii* (ATCC 33618) and mushroom-pathogenic strains of '*P. reactans*' are positive for white line production (Wells et al. 1996). WLIP production provides ecologically important features including solid-surface translocation, surfactant activity, and biofilm formation for the bacterium of origin (Henkels et al. 2014; Rokni-Zadeh et al. 2012). Additionally, WLIP was also reported to be a virulence factor for some pseudomonad strains on the caps of *A. bisporus* (Henkels et al. 2014). The WLIP is produced by taxonomically diverse species of pseudomonads, e.g., *P. fluorescens* (Ghequire et al. 2013), *P. putida* (Rokni-Zadeh et al. 2013), *P. protegens*, and *P. aurantica*

(Mehnaz et al. 2013). Figure 3 shows *P. tolaasii* strains (BP1106, BP1104, and BP1088) that produced the white line when cultured side by side with *P. protegens* (Pf-5), while *Pseudomonas* spp. other than *P. protegens* (BP1107, BP1286, BP1230) were negative for WLIP. Another determinative character for *P. tolaasii* is the production of tolaasin, which can be determined by the amplification of a fragment of the tolaasin-synthetize gene fragment involved in tolaasin biosynthesis (Lee and Cha 1998; Lee et al. 2002).

Siderophore typing (siderotyping) was proposed as a rapid and efficient typing method for discrimination of fluorescent pseudomonads associated with mushrooms (Munsch et al. 2000, 2002b). A significant diversity was observed through the siderotyping of mushroom-associated pseudomonads (e.g., '*P. reactans*' and *P. tolaasii*) (Munsch et al. 2000), which was further confirmed with molecular genotyping among these strains (Munsch et al. 2002b). Interestingly, the *P. tolaasii*-like strains, which were clearly distinguished from the standard *P. tolaasii* strains in their siderotyping scheme, were subsequently assigned to a new species as *P. constantinii* (Munsch et al. 2002a).

Culture media can also be used to distinguish *P. tolaasii* from other blotch pathogens and pseudomonads. KB was originally developed by King et al. (1954) for the detection and differentiation of *P. aeruginosa* by enhancement of fluorescein production. On this medium,

Table 1. Phenotypic characteristics (i.e. LOPAT tests, white line-inducing principle [WLIP], and tolaasin biosynthesis) of bacterial species causing blotch disease on mushrooms^a

Species	Reference strains	LOPAT					Fluorescent pigment	WLIP	Tolaasin biosynthesis	Reference
		Levan	Oxidase	Potato soft rot	Arginine dihydrolase	Hypersensitive reaction				
<i>Pseudomonas tolaasii</i>	ICMP 12833 ^T = NCPBP 2192 ^T = LMG 2342 ^T = ATCC 33618 ^T	-	+	-	+	-	+	-	+	Braun-Kiewnick and Sands 2001
<i>P. constantinii</i>	CFBP 5705 ^T	-	+	-	+	-	+	nd	-	Munsch et al. 2002a
<i>P. agarici</i>	ICMP 2656 ^T	-	+	-	-	+	+	nd	-	Young 1970
' <i>P. gingeri</i> '		-	+	-	+	nd	+	nd	-	Wong et al. 1982
' <i>P. reactans</i> '			+	-	+			+	-	
<i>P. protegens</i>	CHA0 ^T = CFBP 6595 ^T , = DSM 19095 ^T	-	+	-	+	±	+	+	-	Ramette et al. 2011
<i>P. putida</i>	CFBP 2066 ^T	-	+	-	+	-	+	+	-	Braun-Kiewnick and Sands 2001
<i>P. fluorescens</i>								+	-	Champion et al. 1980; Palleroni 1984; Braun-Kiewnick and Sands 2001
Biovars I, II, and IV		+	+	-	+	-	+		-	Stanier et al. 1966
Biovars III and V		-	+	-	+	-	+		-	

^a nd: not determined. '*P. gingeri*' and '*P. reactans*' are invalid names.

many fluorescent pseudomonads including *P. tolaasii* produce characteristic green to deep green colonies covered with whitish membrane-like materials. The white line producing pseudomonads produce pale yellow colonies surrounded by a purple ring, while the non-pathogenic mutant of *P. tolaasii* and non-pathogenic *Pseudomonas* spp. strains, which lost (or do not have at all) the white line reacting activity, produced pale yellow colonies (Suyama et al. 2000). However, the accuracy of these phenotypic tests remains speculative, as the identity of organisms used as positive and negative controls in many studies were themselves dubious. These tests continue to be an important component of polyphasic taxonomic studies of the genus *Pseudomonas*, while none is diagnostic.

The taxonomy of these organisms was further complicated by the separation of species into biological varieties or biovars, which were further strengthened by studies of genetic relationships using RNA-DNA homology (De Vos et al. 1985). As a standalone species, *P. tolaasii* was classified in RNA-DNA homology group I of fluorescent pseudomonads, which is closely related to biovar V of *P. fluorescens* (Table 1; De Vos et al. 1985). Later, DNA-DNA homology became the gold standard for species determination (Palleroni et al. 1972). However, few researchers had the tools (type strains and technical expertise) to identify strains using DNA-DNA homology (Vinatzer and Bull 2009).

DNA sequencing-based methods are now available to readily differentiate species of *Pseudomonas* and are useful in both classification and identification. Mulet et al. (2010) used four housekeeping genes as well as the 16S rRNA gene sequences in a multilocus sequence analysis (MLSA) to study the majority of the available type strains of *Pseudomonas*. Of the two major phylogenetic lineages described by Mulet et al. (2010), to date, all of the bacterial mushroom pathogens have been identified as fluorescent pseudomonads (Fig. 4; Martins, Ramos-Sepulveda et al., unpublished data). Recently, the type strains of *Pseudomonas* species (confirmed organisms representing the species to which the name is associated) were sequenced to help with definitive identifications. Hesse et al. (2018) provided whole genome sequences for all the type strains of 166 validly named species or subspecies of *Pseudomonas* and identified whole genomes of 189 additional unnamed *Pseudomonas* species represented as whole genome-sequence databases. These data are now being used to identify *Pseudomonas* species and isolates causing bacterial blotch as well as playing other roles in mushroom production

(Martins, Ramos-Sepulveda et al., unpublished data). As expected, the majority of strains in commercial mushroom growing facilities in Pennsylvania, U.S.A., are *P. tolaasii*; however, among the pathogens in the region are novel species not yet described.

Although it has been thought that *P. tolaasii* is easily distinguished from *P. fluorescens* strains or other fluorescent pseudomonads by its pathogenicity on mushrooms and the white line reaction (Wong and Preece 1979), the inability of most researchers to conduct the DNA-DNA or DNA-RNA homology tests needed for conclusive identification may have resulted in misidentifications. Discrepancies in the taxonomy of *P. tolaasii* are documented; some strains were renamed as *P. tolaasii* from their original identification as *P. fluorescens* and others were reclassified from *P. tolaasii* to *P. costantinii* (Munsch et al. 2002a; Paine 1919). Recently, researchers have begun to use sequence analysis of individual genes to identify mushroom pathogens from mushroom production regions in New Zealand, Australia, and England (Godfrey et al. 2001). To test whether the strains deposited in international collections (Supplementary Table S1) have been misidentified as *P. tolaasii*, an MLSA was performed with concatenated sequences of four housekeeping genes (*gapA*, *gltA*, *gyrB*, and *rpoD*) using previously published methods (Fig. 4; Bull et al. 2011; Hwang et al. 2005). For the taxonomic analysis presented here (Fig. 4), gene sequences from 163 *Pseudomonas* type strains and representatives of 188 unnamed *Pseudomonas* spp. (Hesse et al. 2018, in blue) were compared to strains identified as *P. agarici*, '*P. gingeri*', and *P. tolaasii* from international collections. Most of the type strains and representative unnamed species are not shown in Fig. 4 because they are not related to the mushroom pathogens evaluated. According to the results shown in Fig. 4, some strains deposited in international collections were misidentified. Of the 55 strains of *P. tolaasii* evaluated, 38 were correctly placed in the same clade as *P. tolaasii* (Fig. 4 clade labeled C1). In addition, one strain previously deposited as *P. agarici* was correctly identified. However, 17 strains deposited as *P. tolaasii* had genetic distances greater than 0.0375 from *P. tolaasii* (Fig. 4), the genetic distance considered to correspond to the species cutoff for *Pseudomonas* analyzed by MLSA (Bull et al. 2011). These 17 strains represent potential unnamed species identified by Hesse et al. (2018). Thus, it is important that researchers use type strains for comparisons when identifying bacterial blotch pathogens and that the identity of previously identified blotch pathogens be questioned. The recently published sequences of type strains and theoretical species will facilitate the identification of mushroom associated pseudomonads. A broader diversity of strains causing blotch were also isolated from oyster mushrooms in Korean facilities (Mu et al. 2015). These isolates were tentatively identified as *P. abietaniphila*, *P. azotoformans*, *P. plecoglossicida*, and *P. brenneri* using comparisons of 16S rDNA sequences. The use of MLSA or comparisons of their whole genomes will help resolve the identity of these strains.

Identification is important for disease management because it is important to ensure that control measures work on the entire diversity of strains causing bacterial blotch. Equally importantly, studies have shown that beneficial and commensal pseudomonads coexist in and on mushrooms and in the casing materials (Frey-Klett et al. 2011; Soler-Rivas et al. 1999a; Wichmann et al. 2008). The presence of *P. putida* and other fluorescent pseudomonads has been shown to promote the primordia development and growth (Noble et al. 2003; Rainey 1991a, b; Saxon et al. 2014). We are attempting to develop management strategies that reduce pathogenic populations of *Pseudomonas* species, while enhancing populations of those that can increase mushroom yields.

Occasionally, blotch-like diseases have been attributed to organisms other than *Pseudomonas* species. For example, Gill and Cole (1992) described a non-fluorescent bacterium, *Pseudomonas cepacia* (later renamed as *Burkholderia cepacia*; Yabuuchi et al. 1992) causing cavity disease (mild blotching to deep pitting, and pervasive cavities extended from the cap surface to the stipe) of *A. bitorquis*. Another bacterium, *Burkholderia gladioli* pv. *agaricicola* which was originally described as *Pseudomonas gladioli* pv. *agaricicola*,

Fig. 3. White line reaction assay using *Pseudomonas fluorescens* (strain Pf-5 in the center of the plate: as white line inducing principle [WLIP] producer) against *Pseudomonas tolaasii* (left side of the plate: BP1106, BP1104, and BP1088), as well as *Pseudomonas* spp. (right side of the plate: BP1107, BP1286, and BP1230), which are closely related to *P. tolaasii*. Black arrows indicate the white line precipitates in the left side of the plate. All bacterial strains were grown on King's medium B at 27°C for 24 h.

is responsible for the soft rot of mushrooms (Gill and Tsuneda 1997; Lincoln et al. 1991). Additionally, several enterobacterial species, i.e., *Ewingella americana* (causing internal stipe necrosis of *A. bisporus*, *Lentinula edodes*, and *Pleurotus ostreatus*), *Serratia liquefaciens*, and *Cedecea davisae* (causing yellow blotch on mushrooms) were described to be capable of infecting mushrooms (Chowdhury et al. 2007; Reyes et al. 2004; Sivanesan 2003).

Isolation of Bacterial Blotch Pathogen(s)

Mushroom growing substrates are rich in organic materials and moisture, and thus support a high density of bacteria. Microbial composition changes dramatically across the different stages of mushroom production, compared with the relatively stable microbiome of outdoor field soil (Fig. 1; Gagliardi et al. 2001; McGee et al. 2017a; Partanen et al. 2010; Pecchia et al. 2014; Vieira and Pecchia 2017). An appropriate selective medium is useful for capturing pathogenic fluorescent pseudomonads from microbially diverse mushroom compost and casing layers. As described above, the KB (*Pseudomonas* agar F) medium has been used for specific isolation of fluorescent *Pseudomonas* species causing blotch on mushroom tissues and may provide some initial differentiation among pathogenic and non-pathogenic strains (Suyama et al. 2000).

Mushroom Microbiomes

Microbial activities strongly influence the mushroom cropping process, and several studies have been targeted at understanding how microbial ecology affects various aspects of the mushroom cultivation system (e.g., composting, pinning, and disease occurrence). The *A. bisporus* cropping process can be divided into two heterogeneous microbial environments, compost and casing (Fig. 1), which may each receive microbial colonizers from different sources. Both environments are colonized by a broad range of bacteria (McGee et al. 2017a; Vieira and Pecchia 2017) and fungi (Carrasco et al. 2019; McGee et al. 2017b; Straatsma et al. 1994a). As is true in many environments, bacterial richness is much higher than fungal richness in the mushroom cropping system; however, fungal biomass during phase II of composting (conditioning phase after pasteurization) is double the bacterial biomass (Sparling et al. 1982; Vos et al. 2017; Wiegant et al. 1992). During composting (phase II), fungi play an important role in carbohydrate (i.e., hemicellulose and cellulose) degradation, which is critical for supporting mushroom growth, and may help to limit competitors and/or pathogens (Kabel et al. 2017; Straatsma et al. 1994a, b; Vos et al. 2017). After spawning, mushroom mycelia become the most substantial component of the microbial biomass, and this appears to impact bacterial and fungal composition in the compost and casing layer (McGee et al. 2017a, b).

Fig. 4. Multilocus sequence analysis of *Pseudomonas* species causing blotch diseases of mushrooms. Concatenated sequences of four housekeeping genes (*gapA*, *gyrB*, *gltA*, and *rpoD*) were used for construction of the phylogenetic tree. The clades and clades represented by singletons are represented respectively by the capital letters C and S, followed by a numeric label. Neighbor joining algorithm was used and Kimura 80 was used for the distance measure. Branching values determined using 1,000 bootstraps. Nodes supported by bootstrap values lower than 100 are labeled and those below 50 were collapsed. *Pseudomonas syringae* pv. *syringae* ICMP 3023T was used as outgroup. The labels for the type strains are presented in blue, correctly named strains from culture collections presented in green, and strains from culture collections that are incorrectly named are presented in black.

A number of bacterial phyla have been identified across the mushroom cropping process, including Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, Deinococcus-Thermus, Fibrobacteres, Firmicutes, Gemmatimonadetes, Hydrogenedentes, Lentisphaerae, Planctomycetes, Proteobacteria, Saccharibacteria, Tenericutes, Thermodesulfobacteria, Thermotogae, and Verrucomicrobia (Kertesz and Thai 2018; Székely et al. 2009; Vieira and Pecchia 2017). Members of the order Pseudomonadales have been commonly detected in both compost and casing using high-throughput sequencing, fingerprinting, and culture-dependent methods (Godfrey et al. 2001; McGee et al. 2017a; Pecchia et al. 2014; Siyoum et al. 2016; Vieira and Pecchia 2017). Some of these bacteria appear to be associated with the development of *A. bisporus* fruiting bodies (Noble et al. 2003; Rainey 1991a, b) as well as mushroom disease (Largeteau and Savoie 2010; Munsch and Alatosava 2002; Soler-Rivas et al. 1999a). Samson et al. (1987), through culture-based approaches, estimated that pseudomonads represented up to 10% and 50% of the colonies they were able to capture from compost and casing, respectively. The primary source of pseudomonads in mushroom farms appears to be the peat moss used as casing material (Qiu et al. 2019; Samson et al. 1987; Wong and Preece 1980). Various *Pseudomonas* spp. strains play important roles in mushroom fructification and the occurrence of blotch disease. Diversity, structure, epidemiology, and pathogen-host interactions of *Pseudomonas* spp. in *A. bisporus* cropping systems is not fully understood. Future investigations should address how compost management techniques influence *Pseudomonas* spp. composition, and link this to production success. Furthermore, relationships between casing materials (peat vs. alternative mixes) and *Pseudomonas* spp. composition, as well as the dispersal of various pseudomonads between compost, casings, and mushrooms, should be investigated for impacts on fruiting body development and production success.

The management of microbiomes in the mushroom production system may present opportunities for managing blotch disease, as being investigated in other plants vs. soils systems (Panke-Buisse et al. 2015). As an experimental tool, microbiome analyses would enable us to 1) transfer yet-to-be-cultured microorganisms, 2) retain some community-level interactions, and 3) select microbiomes based on group phenotypes. As an example of how this latter point might apply to mushroom blotch, microbiomes could be selectively transferred from mushrooms that appear to be disease resistant or susceptible, to a new crop of mushrooms. It would then be possible to determine the extent to which both microbiome composition and disease resistance are reproducible in this system. In addition, the selective manipulation or sterilization of different system components (e.g., casings, compost, and room surfaces) will help to reveal the flow of microorganisms throughout production, as well as likely management targets.

Epidemiology and Environmental Conditions that Promote Blotch Disease

Although some diseases affect mushroom quantity, the main crop loss from bacterial blotch and a number of other mushroom diseases (e.g., cobweb, mushroom virus X, and dry bubble) is attributed to the reduction of product quality and marketability. Another distinction between bacterial blotch and three major fungal diseases of cultivated mushroom (dry bubble caused by *Lecanicillium* spp., green mold caused by *Trichoderma* spp., and cobweb caused by *Cladobotryum* spp.; Błaszczak et al. 2013; Grogan 2006; Ware 1933) is that unlike the non-spore-forming bacterial blotch pathogen(s), fungal spores can quickly spread the fungal pathogens throughout the production room. Blotch causing bacteria are found in the compost and casing everywhere in the room, hence, they do not need to be vectored around the room or in the cap-to-cap distances. Blotch pathogen(s) proliferate only when the climatic conditions are favorable for the pathogen (Nair and Fahy 1973). Some factors predisposing mushrooms for infection include low compost nutrition or low compost fill weight that results in low bed temperatures, which decreases the evaporation rate during irrigation on the mushrooms (Beyer 1993, 2005; Lomax 2007; Wuest 1971).

The epidemiology and development of bacterial blotch is affected by the source of primary inoculum, as well as the ability of the pathogen to grow and multiply under given environmental conditions. Primary sources of *P. tolaasii* in a mushroom house may be the peat and limestone used in the casing process (Wong and Preece 1980). Once the pathogen is established on the mushroom beds, secondary spread of *P. tolaasii* may be from contact with symptomless but infected caps, by the hands, clothing, and shoes of the workers, as well as their picking baskets, knives, and ladders (Wong and Preece 1980). Tap water containing *P. tolaasii* may be an additional source of inoculum; if used for preparation of casing or irrigating, the crop will result in an epidemic of blotch in the first flush (Wong and Preece 1980). When this occurs, it is almost impossible to control the disease under such conditions. Splashing of water spreads the bacteria from the casing material to the young pins, but if cap surfaces are allowed to dry within 3 h, infection may not occur (Royse and Wuest 1980). When the casing is less receptive to water during irrigation, water will remain on the casing surface for a longer period thereby exposing pins and mushrooms to water for extended periods (Beyer 1993, 2005). Furthermore, colonized mushroom spores may transport the pathogen(s), as well as sciarid flies and mites, which are among the common pests of mushrooms (Scheepmaker et al. 1997; Wong and Preece 1980).

Blotch disease incidence and severity are not necessarily related to the pathogen's population in the casing layer; rather, it can be the function of the density of the pathogenic bacteria on mushroom caps, as well as some ecological and microclimatic factors at the mushroom surface (Pecchia et al. 2014; Royse and Wuest 1980; Siyoum et al. 2016). The threshold at which *P. tolaasii* is able to induce blotch symptoms is difficult to estimate because the bacterium may adjust its virulence behaviors depending on the environmental conditions and the surrounding microbiome (Nair and Fahy 1973; Soler-Rivas et al. 1999b; see below). After such a switch, the precise number of pathogenic and non-pathogenic forms is difficult to estimate, and the disease incidence is no longer proportional to bacterial concentration (Soler-Rivas et al. 1999b). The threshold values have been proposed to range from 2×10^5 CFU/cap (corresponding to 7.7×10^4 CFU/cm² of cap surface) to 1×10^8 CFU/cap to trigger disease symptoms, and depends on the environmental conditions, age of the mushroom, and strain of *P. tolaasii* (Nair and Bradley 1980; Nair and Fahy 1972; Olivier et al. 1997; Soler-Rivas et al. 1999b; Wong and Preece 1982). Furthermore, *P. tolaasii* is present in the casing layer of both diseased and healthy beds, in a range of 10^7 to 10^9 cells/g in diseased beds and undetectable to 10^7 cells/g in healthy beds (Grewal and Rainey 1991; Olivier et al. 1997; Wong and Preece 1982). Hence, the threshold of 10^7 cells/g of the pathogen in the casing layer seems to be the minimum population density required to trigger blotch symptoms on the mushroom caps under conducive conditions.

Environmental conditions in the production house, as well as the microclimatic conditions around individual mushrooms, influence pathogen growth (Beyer 1993, 2005). Under moist conditions and fluctuating temperature, the incubation time for the bacteria can be as short as 1 to 2 h and in only 3 to 6 h, symptoms can develop (Beyer 1993, 2005; Royse and Wuest 1980). It has been observed that the bacterial blotch that occurs during the first break is less severe than blotch that occurs during the second break (Navarro et al. 2018; Olivier et al. 1997). It might be associated with mushroom resistance, which is thought to decrease as the nutrients are depleted from the substrate, or that inoculum levels of the pathogens needed to trigger symptoms increase as the crop proceeds; however, the feasible changes in the pathogenicity of bacterial strains within a harvesting cycle remain to be determined. Low compost moistures cause high moisture content in the mushroom tissue that results in free moisture on the surface of the mushroom, even when they are not watered (Beyer 1993, 2005). This higher moisture may favor the pathogens. Any other cultural factors that cause mushrooms to be stressed or contribute to low mushroom respiration/transpiration and low evaporation rates may predispose the crop to blotch (Beyer 1993, 2005).

Pathogenicity Mechanism(s) of the Causal Agent(s)

Tolaasin. Most of the members of *Pseudomonas* spp. devote a considerable part of their genome to synthesize structurally diverse lipopeptides, which are associated with varied functions, i.e., motility, surfactant activity, attachment to surfaces, biofilm formation, and pathogenicity (Frey-Klett et al. 2011; Henkels et al. 2014; Raaijmakers et al. 2010; Reder-Christ et al. 2012; Rokni-Zadeh et al. 2013). Furthermore, several *Pseudomonas* species are known for their antifungal metabolite production in nature. For instance, *P. tolaasii* produces a number of non-specific virulence factors, which evoke disruption of mushroom cell membrane function, resulting in nutrient release (Soler-Rivas et al. 1999a). Lelliott et al. (1966) noted that *P. tolaasii* is a normal constituent of mushroom microflora, but under certain circumstances produces a toxic metabolite that discolors or pits mushrooms. Subsequently, a low molecular weight substance stable at high temperatures and pH ranging between 3.0 and 9.0 and caused mushroom cap browning, was detected in the cell-free culture filtrate of *P. tolaasii* (Nair and Fahy 1973) and named 'tolaasin' (Lelliott et al. 1966; Nair and Fahy 1973).

Tolaasin is an 18-amino acid lipodepsipeptide that forms ion channels and acts as a biosurfactant to disrupt the plasma membrane of mushroom cells, allowing *P. tolaasii* access to cell nutrients (Nutkins et al. 1991). Molecular structure, categorization, and physiological/biological characteristics of tolaasin were reviewed previously (Soler-Rivas et al. 1999a). Tolaasin activity can be detected by inoculating a bacterial suspension or cell-free culture extract on potato slices, resulting in blackening of the slices in the presence of the toxin at concentration of 4 ppm (Shirata et al. 1995). Furthermore, tolaasin-producing strains were shown to lyse horse, sheep, and bovine erythrocytes, although no detrimental effect of the toxin on human health was reported (Brodey et al. 1991; Munsch and Alatossava 2002; Nutkins et al. 1991; Rainey et al. 1991). A 2.3 kb DNA fragment is involved in tolaasin production, and the Tol-mutant is negative for pathogenicity on mushrooms, white line test, hemolysis test, as well as the high-pressure liquid chromatography (HPLC) analysis of the culture filtrate (Lee and Cha 1998). No other mushroom pathogen has been reported to produce tolaasin (Lee et al. 2002).

Furthermore, other toxins have been found to cause discoloration and blotch symptoms in mushrooms. For instance, the antifungal metabolites 2,4-diacetylphloroglucinol and pyoluteorin, produced by the soil bacterium *Pseudomonas protegens* Pf-5, has been shown to cause lesions on *A. bisporus* (Henkels et al. 2014), though this organism has not yet been found in mushroom production. It is not known what toxins might be produced by the broad diversity of pathogenic strains recently isolated (described above), which is the work of ongoing investigations. However, understanding the structure and modes of action of these virulence factors will allow us to target them for control of bacterial blotch. As a control strategy, for example, it has been shown that the application in mushrooms of certain food additives acts as a tolaasin-inhibitory factor (TIF), suppressing brown blotch disease caused by *P. tolaasii*. TIFs interact with tolaasin and inhibit the toxin activity on the mycelial membrane (Yun et al. 2017).

Control

The major constraint in managing bacterial blotch is that the casing layer (thought to be an important source of primary inoculum; Fig. 1) cannot be treated with many of the commercial broad spectrum antibiotics and chemicals in the mushroom-growing process, because some of the bacterial species present in the casing layer (e.g., *P. putida*) are necessary for promoting primordia development and growth (Fig. 1; Fletcher and Gaze 2007). Although a range of abiotic parameters (temperature, CO₂ concentration, relative humidity, and pH) were shown to influence sporophore (mushroom cap or fruiting body) production (Flegg and Wood 1985), the major stimulus is provided by pseudomonads (e.g., *P. putida*) in the casing layer (Noble et al. 2003; Rainey et al. 1990, 1991a, b). *P. putida* markedly increases hyphal growth and morphology of *A. bisporus* only when it establishes direct contact with the mycelium in the casing soil, while *P. tolaasii* survives well in the same environment (Rainey 1991a, b). Hence, several attempts have been made to develop an

appropriate method to selectively control the bacterial blotch pathogen without affecting *P. putida* population (Wong and Preece 1985a, b). Switching between the pathogenic and non-pathogenic forms of *P. tolaasii* occurs due to the reversible duplication of a 661-bp element within the *pheN* locus (Sinha et al. 2000), similar to other two-component systems of regulation (Grewal et al. 1995), which are easily mutated by subculturing on rich media (Bull et al. 2001). Hence, the control method should reduce or eradicate both the pathogenic and non-pathogenic forms of the pathogen on the mushrooms and the substrate. A range of approaches is currently being used to combat the bacterial blotch pathogen(s) on farms. Among them, precise management of relative humidity, temperature, and ventilation in the rooms, treatment with chemicals, development of blotch-resistant mushroom strains, as well as biological control using the mushroom-associated bacterial strains are the most promising methods.

Managing relative humidity, temperature, and ventilation in the house. Control of bacterial blotch can be achieved by ventilation and temperature management in the mushroom growing houses, which will result in drying the surface of mushroom caps, preventing the growth of *P. tolaasii* to the level that triggers symptoms. Variations on the role of temperature and humidity in the blotch disease management have occasionally been reported in the literature (Gandy 1967; Sinden 1971). Other techniques include water additives to reduce the bacterial population on the surface of the mushrooms (see below). However, even with water treatments, if the surface of the mushroom remains wet for an extended period (>3 h), the bacteria can reach population levels that will cause blotch (Beyer 1993, 2005). Temperature control includes maintaining a steady temperature during the harvesting period, in order to avoid fluctuating air temperatures that may cause moisture condensation on the surface of the mushrooms (Beyer 1993, 2005; Lomax 2007). Condensation on mushrooms may be caused by other environmental factors (e.g., moist air rising up from the compost and through the casing layer and condensation in the cooler microclimate at the surface of the mushroom caps). Moisture from mushroom transpiration needs to be removed from the room (Beyer 1993, 2005; Lomax 2007). Air circulation, even under saturated air conditions, will result in some drying of the surface of a cap. The difference between the mushroom's cap temperatures to the microclimate air temperature must be maintained, and the cap temperature must be slightly warmer than the air temperature. The air exchange per hour as related to the drying ability is important to keep the cap surface dry (Ayers and Lambert 1955; Wuest 1971). Moist air used to dry the mushrooms must be exhausted. Hence, for the first few hours after watering, it is suggested that high volumes of fresh air be introduced into the room to force the moist air out. Although exhaust fans could be used, they may create a condition that draws 'dirty' air into the room. Regardless of how moist air is removed, optimal CO₂ levels may need to be compromised for a few hours, while simultaneously maintaining air temperature as steady as possible. Measuring absolute or relative humidity may improve the grower's knowledge of the drying conditions in a crop. Lomax (2007) reviewed dew point measuring and management in the houses. Growers need to maintain a balance between feathering/scaling (which is caused by excessive air movement within the house) and bacterial blotch occurrence; that is, the balance between too dry and not dry. During cropping, a relative humidity of 85% and an air speed of less than 2 cm/s will result in blotch development, while air speeds higher than 5 cm/s can lead to scaling depending on relative humidity (Fletcher and Gaze 2007). Growers need to determine which relative humidity/air flow combination will scale or blotch mushrooms (Lomax 2007). This concept has been named the Lomax Quadrant (Fig. 5; Lomax 2007; Navarro et al. 2018).

Other control issues include 'pinning' depth, harvesting technique, cleanliness, and post-harvest handling (Beyer 1993, 2005). Pinning describes a stage in the mushroom growing process that includes the formation of recognizable immature mushroom pileus (initials) from fungal mycelium through the casing layer (Fig. 1). The mushrooms should be pinned on the casing surface, as opposed to below the surface, to minimize contact of the casing with the mushroom pins as they develop. Once harvesting begins, hands and gloves should be

as clean as possible and clean picking containers should be used. After harvest, handling of the mushrooms must be minimized, and the mushrooms should be cooled as quickly as possible. Excessive bruising will release sugars and moisture on the mushroom, which will encourage bacterial growth. Fluctuating storage and transport temperatures post-harvest may cause condensation and subsequent blotch development post-harvest (Beyer 1993, 2005; Fletcher and Gaze 2007; Lynch 2018).

Treatment with chemicals. Due to the restrictions in the application of synthesized chemicals on the mushroom beds, chemical treatments are being further disregarded with the mounting of organic mushroom production around the globe, which is the case in many of other agricultural products (Lamichhane et al. 2018). Since the earliest reports on bacterial blotch (Tolaas 1915), chlorination of water with sodium hypochlorite supplied onto the mushroom beds was the widely used preventive chemical control method (Ayers and Lambert 1955; Paine 1919), although it was not very effective (Geels 1995; Geels et al. 1991), with high concentrations often causing browning of mushroom caps (Kleuskens 1983; Royse and Wuest 1980). It is advisable to chlorinate all water used on the casing and watering, although there is no permission to use chlorinated water on the food surfaces in some countries, i.e., those in the European Union. A low concentration of chlorine (≥ 150 mg/liter; Fahy and Bradley 1987) is needed to eliminate the blotch pathogen(s) in most water sources (Royse and Wuest 1980). Even if using chlorinated water, the timing of watering is a fundamental factor influencing disease development (Wong and Preece 1980). Stabilized chlorine dioxide was reported to have greater effect than sodium hypochlorite (Markowitz and Bodine 1989), and a combination of 50 ppm of stabilized chlorine dioxide and 0.25% of calcium chloride was determined to be the best mixture to control bacterial blotch (Soloman et al. 1991). Neither chlorine dioxide nor calcium chloride had significant effects on yield, but postharvest quality and shelf life were significantly improved (Soloman et al. 1991). Bacterial counts, browning, as well as senescence were significantly reduced with the use of the combination. However, calcium chloride, whether added separately or in combination with chlorine dioxide, can reduce the yield of off-white hybrid mushrooms (Soloman et al. 1991). The population levels of *P. tolaasii* recovers easily from a single chlorine application directly after the first flush. Repeated treatments with chlorinated water seem unavoidable to obtain any significant reduction in blotch (Geels 1995). Ayers and Lambert (1955) reported the usefulness of successive watering with up to 100 $\mu\text{g}/\text{ml}$ of active chlorine for this purpose.

Other chemical treatments have been evaluated. Wong and Preece (1985b) evaluated the bactericidal activity of six disinfectants and their toxicity to mushrooms and noted that the bactericidal activity of 2-bromo-2-nitropropane-1,3-diol (bronopol) against *P. tolaasii* is enhanced by the addition of calcium carbonate, tween 80, EDTA, and phenylethanol (Wong and Preece 1985a, b, c). Sterility could be achieved in 24 h, with a single application of bronopol at 100 $\mu\text{g}/\text{ml}$

in tap water during routine watering (Wong and Preece 1985b, c). Furthermore, 1% kasugamycin (an antibiotic produced by *Streptomyces kasugaensis*) treatment significantly reduced total blotch incidence to only 9%, in comparison to the untreated mushrooms with 57% blotch infection, although the use of kasugamycin in mushroom houses is not approved in the U.S. (Geels 1995). In some cases, plant-based substrates and extracts were also investigated for their potential control capability. For instance, it has been shown that the presence of *Citrus limon* (lemon) and *Salvia miltiorrhiza* (red sage) plant debris in the compost decreased the incidence of bacterial blotch and the number of diseased mushroom fruit bodies (Dawoud and Eweis 2006). Several other chemicals, disinfectants, and antibiotics have been studied for their potential to control bacterial blotch, but none has been found to be fully effective and non-toxic to the crop or consumers (Munjal et al. 1989; Vantomme et al. 1989). Hence, the development of resistant mushroom strains, as well as biological control, remain the most promising approaches to combat bacterial blotch.

Development of resistant mushroom strains. Selection and breeding for resistance to brown blotch is a durable and cost-effective strategy, which could provide new mushroom genotypes less susceptible to the pathogen, thus decreasing crop losses (Moquet et al. 1999). It has been suggested that a broad diversity of pathogens causing blotch should be considered and evaluated when mushroom strains are screened for resistance. The methods used for plant breeding and genetic screening could be applied to edible mushrooms (Moquet et al. 1999). Early screens of mushroom cultivars against bacterial blotch were conducted in France, which showed that the wild strains of *A. bisporus* collected in France were significantly more resistant than the commercial strains (Olivier et al. 1997). Resistance to tolaasin could be considered as the preliminary assay of a candidate mushroom cultivar for bacterial blotch resistance; however, it must be complemented by the test of resistance to living bacteria (Moquet et al. 1999) and other toxins that cause blotch symptoms. As a reaction to tolaasin, the resistant mushroom cultivars develop an impenetrable plug of modified or dead hyphae beneath the point of infection, which prevents sporophore invasion by the bacterium (Cole and Skellerup 1986). It has been shown that the traditional brown strains are less susceptible than off-white hybrid strains (Olivier et al. 1997). Due to the genetic linkage between the mushroom cap color and resistance to bacterial blotch, it has been suggested that mechanisms of melanin biosynthesis for natural and pathogen-induced browning are strongly linked together. Hence, these two traits (cap color and resistance to *P. tolaasii*) should be studied together in breeding programs (Olivier et al. 1997). Moquet et al. (1999) observed that a QTL (quantitative trait locus), which is strongly linked to the cap color forming PPC1 (pilei pelles color) locus in *A. bisporus*, is responsible for the resistance to *P. tolaasii*. The strong linkage detected by interval mapping between the resistance QTL and the PPC1 gene suggests that it might be the PPC1 gene itself (Moquet et al. 1999). Recently, CRISPR-Cas9 was used to generate *A. bisporus* lines that are resistant to enzymatic browning in mushrooms (Waltz 2016). Polyphenol oxidase (PPO) is one of two enzymes from the mushroom that causes browning (Weijn et al. 2013). Yinong Yang (unpublished data) used genome editing to create a mutation in one of six genes required for PPO synthesis, reducing the browning activity by 30%. However, it is not known whether a mutant resistant to browning will also be resistant to blotch symptom development due to reduced sensitivity to toxins produced by the pathogens. Genome editing will be a valuable tool for the development of lines resistant to blotch. Recently, Sonnenberg et al. (2017) reviewed the developments made in breeding of cultivated mushroom, although the studies conducted on bacterial blotch resistance are far less numerous than those conducted on quality-related traits (Moquet et al. 1999; Sonnenberg et al. 2017).

Biological control. Special characteristics of mushroom cultivation, with environmentally controlled production facilities and the rapidity of the cropping cycle, create an excellent opportunity to deploy a biological control system. Indeed, biological control is favored by the special characteristics of mushroom cultivation facilities, e.g.,

Fig. 5. Lomax Quadrant, described by Dr. Ken Lomax (2007), re-illustrated.

ecologically stable conditions, high relative humidity, and accessibility of free moisture and nutrition for the biocontrol agents. Furthermore, transition from vegetative growth to reproductive phase in the cultivated mushroom is stimulated by application of a mixture of peat and lime (casing layer) to the surface of colonized compost (Fermor and Lynch 1988; Rainey 1991a, b). Hence, the 'casing' provides a feasible opportunity to add the biological control agents to the mushroom substrate. *P. tolaasii* was found in the casing layer of both diseased and healthy beds, whereas the pathogen was detected only in the compost of diseased mushroom beds, revealing that the bacterium is among the natural microbial flora of the casing layer (Nair and Fahy 1972). Hence, targeting the casing layer with the biocontrol agents is likely to result in the control of the blotch pathogens. Among the different substrates tested as a carrier for the antagonists (i.e., autoclaved sphagnum peat, gamma-irradiated peat, and pasteurized spent compost), gamma-irradiated peat with a moisture content of 50 to 60% (wet weight) and an incubation period of 4 to 5 weeks at 25°C best favored the growth and survival of bacterial antagonists (Nair and Fahy 1976).

Different strategies have been proposed to develop a sustainable biocontrol method to be used in the mushroom production. Table 2 summarizes antagonistic microorganisms and bacteriophage used for the biological control of bacterial blotch and associated research. Purification, enrichment, and application of antagonistic microorganisms associated with mushroom beds was the first approach, which started in the early 1970s (Fahy et al. 1981; Nair and Fahy 1972). Subsequently, application of pathogen-specific bacteriophages resulted in an acceptable disease control at least under laboratory conditions (Kim et al. 2011; Munsch and Olivier 1995). We are not aware of these being used commercially in the U.S.

Antagonistic microorganisms. Development of an effective and durable biocontrol method requires a series of screening assays to determine the mechanisms by which the candidate antagonists (i.e., antibiotic, siderophore, and bacteriocin production, carrying bacteriophages, as well as predatory activity) function. Nair and Fahy (1972) investigated the use of antagonist bacterial strains for bacterial blotch control in mushrooms produced in Australia. Biological control with *Burkholderia multivorans* (at that time: *Pseudomonas multivorans*), *P. fluorescens*, and *Enterobacter aerogenes* isolated from the casing layer and mushroom caps was more effective than either sodium hypochlorite (150 µg/ml free available chlorine) or formalin (0.25% v/v) applied with regular watering. Combining biological control and sodium hypochlorite was the most effective treatment. The chemicals were applied at every watering following casing (a total of 12 applications), and the biocontrol agent was applied at the days 0, 15, 34, and 46 while not applied until 2 to 24 h post chemical treatments (Fahy and Bradley 1987). Following field scale success, promising antagonistic strains (i.e., *P. fluorescens* and *Burkholderia multivorans*) were commercialized in Australia (patented as 'Conquer' Mauri Foods, North Ryde, NSW, Australia) (Fahy and Bradley 1987; Fahy et al. 1981; Nair and Fahy 1976). However, the efficiency and commercial success of 'Conquer' has not been reported in literature.

Further studies were performed in France (Olivier et al. 1978), Taiwan (Liao et al. 1980; Tu and Liao 1989), and the United Kingdom (Fermor and Lynch 1988). A large-scale commercial pilot study for the biological control of bacterial blotch was conducted in France in 1995 (Miller and Spear 1995). Antagonistic bacterial strains were mainly isolated from the compost and casing layer, although plant materials (e.g., wheat straw) occasionally were used for the isolation

Table 2. Antagonistic microorganisms and bacteriophage strains used for the biological control of bacterial blotch of mushroom, and their modes of action on mushroom substrate

Mode of action	Species	Strains	Source of isolation	Status	Reference
Competition	<i>Burkholderia multivorans</i>	A2	Soil	Commercialized as Conquer/ not present in the market	Nair and Fahy 1972
Competition	<i>Pseudomonas fluorescens</i> / non-pathogenic <i>P. tolaasii</i>	C12	Casing peat	Commercialized as Conquer/ not present in the market	Nair and Fahy 1972
Competition	<i>Enterobacter aerogenes</i>	C10	Casing peat		Nair and Fahy 1972
Competition	<i>Pseudomonas</i> sp.	Bi1	<i>Agrocybe</i> sp.	in vivo	Aslani et al. 2018
Competition	Non-pathogenic <i>P. tolaasii</i>				Olivier et al. 1978
Competition	<i>P. fluorescens</i>				Olivier et al. 1978
Not determined	<i>Kocuria</i> spp.		Wild mushroom	in vitro	Namazi et al. 2016
White line inducing principle (WLIP)	<i>Pseudomonas reactans</i>	SPC 8903	Not indicated	in vitro	Soler-Rivas et al. 1999b
Predator	<i>Bdellovibrio bacteriovorus</i>			in vitro	Saxon et al. 2014
Tolaasin detoxification				in vitro	
Tolaasin detoxification	<i>Mycetocola tolaasinivorans</i>	OM-F11	<i>Pleurotus ostreatus</i>	in vitro	Tsukamoto et al. 2002
Tolaasin detoxification	<i>Mycetocola lacteus</i>	OM-A1	<i>Lepista nuda</i>	in vitro	Tsukamoto et al. 2002
Tolaasin detoxification	<i>Sphingobacterium multivorum</i>	OM-A8	<i>Clitocybe clavipes</i>	in vitro	Tsukamoto et al. 2002
Tolaasin detoxification	<i>Pedobacter</i> sp.	OM-E81	<i>Clitocybe</i> sp.	in vitro	Tsukamoto et al. 2002
Tolaasin detoxification	<i>Acinetobacter</i> sp.	OM-H10	<i>Tricholoma matsutake</i>	in vitro	Tsukamoto et al. 2002
Tolaasin detoxification	<i>Bacillus pumillus</i>	OM-F6	<i>Coprinus disseminatus</i>		Tsukamoto et al. 2002
Bacteriophage	<i>Siphoviridae</i>	φPto-bp6g	Sewer waters in the mushroom room	in vivo	Nguyen et al. 2012
Bacteriophage	<i>Podoviridae</i>	Bf7	Sporocarps of <i>Pleurotus ostreatus</i>	in vivo	Sajben-Nagy et al. 2012

of antagonists (Fermor and Lynch 1988). Beside *P. fluorescens* and *P. putida*, which are commonly used as biocontrol agents, non-pathogenic *P. tolaasii* strains were shown to have antagonistic activity against the bacterial blotch pathogen (Fermor and Lynch 1988; Olivier and Guillaumes 1981; Olivier et al. 1978), although the exact taxonomic position of the later strains remains to be understood. Furthermore, Fermor and Lynch (1988) isolated bacterial strains (mainly pseudomonads) from mushroom growing substrates. After preliminary in vitro screening, 10 strains were evaluated for their ability to suppress the bacterial blotch in vivo on *A. bisporus*, and finally only the strain An195 was used to develop an effective large-scale biological control method (Fermor et al. 1991).

Different methods were proposed for the evaluation of antagonistic activity of bacterial strains (Fermor and Lynch 1988, Tsukamoto et al. 1998). Isolation and preliminary evaluation of putative biocontrol bacterial agents can be performed using a two-step procedure described by Fermor and Lynch (1988). In brief, a freshly prepared suspension of *P. tolaasii* is spread on King's B medium and air-dried. A single colony of the suspected antagonist strain is then spotted on the surface of the medium. Those strains, which produced a clear zone on the bacterial lawn, could be further tested using the inhibition zone method (Fermor and Lynch 1988; Geels and Schippers 1983). The diameter of the inhibition zone around the test strains can be proportionally correlated with the antagonistic activity of the strains (Fermor and Lynch 1988).

As with other forms of biological control, effectiveness of the antagonistic bacterial strains in preventing development of bacterial blotch in commercial farming operations was low compared to the expectations of in vitro suppression of the causal agent (Tsukamoto et al. 1998). The discrepancy may be due to numerous factors, e.g., antagonist populations may decline after application. This could be attributed to the presence of detrimental host components against the antagonist strain, inhibitory components in the casing microbiome, predators of the antagonist (phage), and the limitation of nutrients available for the bacterium on the host mycelia. The pathogen may also exist in a physiological state that may be more tolerant to the control mechanisms within the host environment than in culture media. Low efficiency of protection could also be due to the enhancing of virulence determinants in the pathogen by host signals (Murata and Magae 1996; Russo et al. 2003), or physical competition between the two symbionts on the host (Tsukamoto et al. 1998). Isolation and enrichment of endofungal bacterial strains from mushroom bodies may improve the efficiency of blotch biocontrol and fix the inconsistencies among the in vivo tests. Recently, Aslani et al. (2018) evaluated the biocontrol activity of endofungal bacterial strains recovered from wild mushrooms at various locations of western Iran. Among the 66 bacterial strains, the strain Bi1 which was isolated from *Agrocybe* spp. and identified as a close relative of *Pseudomonas taiwanensis*, was able to reduce blotch symptoms by 96% in vivo. Factors such as the ratios of antagonist/pathogen and timing of application are critical in determining the protection efficiency (Tsukamoto et al. 1998). The extent of biocontrol achieved was similar when the antagonistic agent was added within 4 days before or simultaneously with *P. tolaasii* (Tsukamoto et al. 1998). However, the antagonistic activity of *Mycetocola tolaasinivorans* (strain 9405) clearly decreased when the mushroom fruiting bodies were inoculated with the strain 9405 more than 4 days before the pathogen inoculation (Tsukamoto et al. 1998).

Inconsistencies in commercial application may be alleviated through a better understanding of the mechanisms by which antagonists function (Fravel 2005; Grewal and Rainey 1991; Rainey 1991a, b), understanding of the diversity of the pathogens causing blotch, and their sensitivity to the various methods of antagonism. Different strategies are described by which antagonistic microorganisms combat the pathogen in mushroom beds or on the caps. Among them, displacement of the pathogen with competition for space and nutrients (Olivier and Guillaumes 1981; Olivier et al. 1978), predatory activity against the pathogen either directly (Saxon et al. 2014) or by bacteriophage (Kim et al. 2011), detoxification of tolaasin (Tsukamoto et al. 1998, 2001, 2002), and bacteriocin production (Parret et al. 2005) are considered as the most important modes of actions in

bacterial blotch biocontrol (Savoie et al. 2016). Some of these strategies may benefit from a complete understanding of the diversity of *Pseudomonas* species that cause bacterial blotch because, as demonstrated below, they have a limited spectrum of activity. Despite the broad diversity of pathogens causing blotch, biological control of *P. tolaasii* has been the primary target in part because the pathogen diversity is only now being described. Targeting one pathogen among many may leave mushroom crops unprotected against the overlooked pathogens.

Competition as a biological control strategy. Competition between microorganisms will be increased if they use the same kind of substrates and have similar environmental requirements (Fermor and Lynch 1988). This strategy was applied in France, where non-pathogenic *Pseudomonas* spp. strains isolated from mushroom farms were used to control pathogenic strains of *P. tolaasii* (Olivier and Guillaumes 1981; Olivier et al. 1978). When the competition for space and/or nutrients is the main antagonism strategy, a three-step application schedule (i.e., in casing, pre-pinning, and post first flush times) is the best application method (Fermor et al. 1991). This schedule fits with normal cropping practice and allows the antagonist to be present before *P. tolaasii*. However, if only a one-step application of the antagonist is desired, it is proposed that application at the casing time will provide the best disease control (Fermor et al. 1991). It is important to note that the antagonists must be present before the appearance of the first sporophores, because once the disease is established on the mushroom pins, the treatments have very little effect on the population of the pathogen (Fermor et al. 1991). It has also been proposed that the antagonist must be applied at the end of composting (before spawning) as soon as the compost surface temperature drops to 30°C (Fahy and Bradley 1987). However, other scientists noted that if competition is the primary mode of action, early application of antagonists at spawning could be beneficial (Fermor et al. 1991; Wong and Preece 1980).

Antagonistic bacteria in association with soil dwelling organisms were also reported to decrease the bacterial blotch symptoms on mushroom. For instance, '*P. reactans*' is mostly known as a non-pathogenic saprophyte associated with the compost, casing layer, and mushroom caps. On the other hand, saprophytic (free-living) nematodes such as *Caenorhabditis elegans* ingest the bacterial population of the mushroom bed (i.e., '*P. reactans*'), but do not attack the mushroom mycelium (Wuest and Bengtson 1982). Mass-produced bacterivorous *C. elegans* nematode significantly reduced the intensity of blotch symptoms on mushroom caps. Reisolation of bacterial flora from *C. elegans* gut, which was recovered from mushroom caps, revealed the presence of '*P. reactans*' strains, all of which were antagonists against *P. tolaasii*. *Caenorhabditis elegans* prefers '*P. reactans*' rather than *P. tolaasii* as a food substrate, and possibly spreads the antagonist onto the mushrooms, which may contribute to the suppression of bacterial blotch (Grewal 1991). However, application of '*P. reactans*' could be challenging on mushroom caps due to the counterexamples in the literature regarding its pathogenicity on mushrooms. For instance, Wells et al. (1996) described light brown discoloration on mushroom tissues caused by '*P. reactans*' strains isolated in Pennsylvania. Furthermore, '*P. reactans*' strains isolated from symptomatic *A. bisporus* fruit bodies in Finland were reported to be weakly pathogenic on mushrooms (Munsch et al. 2000). Currently, genome sequence-based taxonomic studies are being completed to properly classify '*P. reactans*' and determine whether all of the previously mentioned pathogenic strains are the same species (Martins et al., unpublished data).

The mechanism of antagonism by pseudomonads against *P. tolaasii* is at least partially due to the production of an iron-chelating siderophore. Henry et al. (1991) showed that a significant decrease in the clearing zone diameter was observed in a bacterial blotch biocontrol assay when iron was added to the culture medium. However, for the majority of antagonists, some other modes of action were also involved. For instance, the greatest disease reduction was obtained when the siderophore-producing antagonists showed antibiotic activity as well (Henry et al. 1991). Fermor et al. (1991) noted that although siderophores could be involved in the action of some antagonists, their

production does not appear to be essential for successful biocontrol. The availability of iron (III) declines 1,000-fold for each pH unit increase (Henry et al. 1991), and thus the siderophore-mediated disease suppression would be greater under neutral or alkaline conditions. Hence, a casing layer with a pH of 6 to 8.5 is the best environment for antagonism in mushroom beds (Flegg and Wood 1985).

Predatory strategy. Mushroom caps treated with Gram-negative predatory bacterium *Bdellovibrio bacteriovorus* (strain HD100) 30 min before *P. tolaasii* 2192^T inoculation developed significantly less blotch symptoms in comparison with the caps inoculated with the pathogen alone (Saxon et al. 2014). *B. bacteriovorus* is a small deltaproteobacterial predator that has evolved to attack, kill, and digest other Gram-negative bacteria in soil and water environments (Negus et al. 2017). The bacterium has a broad host range and kills many antibiotic-resistant clinical pathogens in vitro (Saxon et al. 2014). Reduction in *P. tolaasii* population and disease symptoms was due to the predatory activity of *B. bacteriovorus*. However, like all biological control methods, it is not known if the predator is able to prey on all blotch pathogens. Once attacked, bdelloplasts (rounded dead *P. tolaasii* bodies in which *Bdellovibrio* establishes) grow and replicate within the prey bacterial cell (Saxon et al. 2014). New investigations showed that the predator *B. bacteriovorus* is not harmful for human health and can control pathogens inside living animals (Negus et al. 2017; Tyson and Sockett 2017). However, regarding the broad host range of *B. bacteriovorus*, the application of the bacterium can lead to the distortion of beneficial microbial population (e.g., *P. putida*) in the casing layer, which is pivotal for pinning initiation and sporocarp development (Rainey 1991a, b). Hence, further studies are needed to decipher whether *B. bacteriovorus* is appropriate for the biological control of the bacterial blotch pathogen. The mechanisms of *B. bacteriovorus* predation and the physiological properties that would influence its survival has been reviewed recently (Negus et al. 2017).

Inactivation/detoxification of tolaasin. Various bacterial species capable of detoxifying tolaasin are associated with fruit bodies of edible mushrooms and wild *Agaricus* fungi (Tsukamoto et al. 1998, 2001). A Gram-positive bacterium *Mycetocola tolaasinivorans* (*Actinomycetales*, *Microbacteriaceae*) was isolated from a rotting *P. ostreatus* fruiting body. The bacterium is not pathogenic on *P. ostreatus* and is able to inactivate tolaasin. The advantage of using *M. tolaasinivorans* (strain 9405) is that in contrast to some *Pseudomonas* strains, which produce various antifungal compounds and secondary metabolites that can cause browning on the mushroom, the strain 9405 does not produce any antifungal metabolite (Corbell and Loper 1995; Laville et al. 1992). However, recently we have isolated *Mycetocola* spp. from common mushroom possessing pale brown and tan spot symptoms on the caps. The *Mycetocola* spp. strains were shown to induce pitting symptoms on the cut cap of common mushroom, while no tolaasin detoxification activity was observed when they were co-inoculated with *P. tolaasii* on mushroom caps (E. Osdaghi, unpublished data).

Bacteriocin production strategy of biological control. Despite the multiple biocontrol strains that exhibit antagonistic activity against *P. tolaasii*, no systematic screen of antagonistic strains has been pursued to identify those where inhibition was bacteriocin-mediated. The term bacteriocin is broad and encompasses several distinct proteinaceous narrow-spectrum toxins that are bacterially produced and tend to target strains closely related to the producer (Fett et al. 1995; Riley and Wertz 2002). Pseudomonads, including those commonly found in mushroom production systems, often encode for one or more distinct bacteriocins, typically including a bacteriophage-derived bacteriocin (tailocin), as well as several other single-protein (s-type) bacteriocins (Ghequire and De Mot 2014). For instance, *P. protegens* Pf-5 encodes two distinct plant lectin-like bacteriocins, both of which are active against at least one isolate of *P. tolaasii* (Parret et al. 2005), though this species may also be pathogenic to mushrooms (Henkels et al. 2014). Because bacteriocins are specific in their targeting, it is common for strains within a given species to exhibit variability in both bacteriocin encoding potential, as well as bacteriocin sensitivity scheme. Thus, additional efforts to

isolate and characterize bacteriocins that rely on distinct receptors for targeting, and that exhibit distinct modes of action, produced by strains that can be combined into a single consortium may provide effective control that is robust to the evolution of control-breaking resistance.

WLIP-based biological control strategy. As described previously, Wong and Preece (1979) described the ‘white line test’ using ‘*P. reactans*’ as a specific and reliable method to identify *P. tolaasii*, although others (Goor et al. 1986; Munsch and Alatossava 2002) have questioned the specificity of this test; sequence-based identification methods and whole genome sequences should help to resolve this (Martins, Ramos-Sepulveda et al., unpublished data). The white line reaction is the result of a specific interaction between two diffusible lipodepsipeptides, tolaasin and WLIP (Mortishire-Smith et al. 1991). White line reaction is structure-specific, and it cannot be triggered by *Pseudomonas* strains producing other lipopeptides and is not elicited by WLIP analogues with minor structural differences such as viscosin (fifth amino acid residue L-Leu instead of D-Leu) or viscosinamide (viscosin variant with D-Gln instead of D-Glu at position 2) (Rokni-Zadeh et al. 2011). The WLIP non-ribosomal lipopeptide synthesis system of ‘*P. reactans*’ LMG 5329 was identified and shown to be similar to the *P. fluorescens* SBW25 viscosin system but remarkably different from the WLIP-generating system previously identified in the rice rhizosphere isolate *P. putida* RW10S2 (Rokni-Zadeh et al. 2013). A cell-free crude extract containing WLIP was reported to inhibit bacterial blotch pathogen at least 4 days after application at 4 to 7°C (Soler-Rivas et al. 1999b). The concentration of the crude WLIP necessary for control of blotch pathogen is reduced when the pathogen population is low (Soler-Rivas et al. 1999b). The precise mechanism of the inhibition of blotch symptoms by WLIP is unknown. Further investigations are needed to shed a light on the possibility of applying WLIP at a farm scale to control the bacterial blotch.

Phage therapy. Initially described almost a century ago (d’Herelle 1931), bacteriophages are small viruses displaying the ability to reproduce inside the host bacterial cells and kill them while they do not affect cell lines from other organisms (Wittebole et al. 2014). Once the bactericidal property of bacteriophages was discovered, their application against infectious bacterial species (phage therapy) was hypothesized (Adams 1959; d’Herelle 1931). Investigations on the suitability of bacteriophages as biological control agents in agriculture have significantly increased since the beginning of the current century, and several successful attempts were reported on the control of bacterial diseases of vegetables and annual crops (Svircev et al. 2011). Jones et al. (2007) reviewed the use of bacteriophages as agricultural biopesticides and commercialization. Since bacteriophages can rapidly lyse and kill host bacteria with a high host specificity, they have been found to be an efficient alternative to conventional chemical therapies (Matsuzaki et al. 2005). Phages can amplify from a small initial population and persist in the environment while the host bacterium is amplifying. Lytic activity enables phage population to grow to match the abundance of host bacteria in their habitat (Nguyen et al. 2012). It is hypothesized that bacteriophages with high adsorption rate, short latent period, and large burst size will provide more success with phage therapy (Merabishvili et al. 2018).

Munsch et al. (1991) used the bacteriophage TO1 isolated from *A. bisporus* against strains of *P. tolaasii*. Among the eight independent experiments, the bacterial blotch control ranged from 30 to 80% (Munsch et al. 1991). The use of TO1 phage was compatible with most of the registered pesticides in mushroom farms at that time (Munsch and Olivier 1995). Recently, Kim et al. (2011) screened bacteriophages of *P. tolaasii* for biological control of bacterial blotch disease, and bacteriophage ϕ Pto-bp6g (Table 2) was shown to have strong bactericidal activity against the pathogen (Nguyen et al. 2012). The whole genome sequence of phage ϕ Pto-bp6g showed that it could be potentially applicable as a safe biological control agent against bacterial blotch disease in mushroom cultivation (Nguyen et al. 2012). Lytic phage BF7 was also isolated from smashed sporocarps of oyster mushrooms (*P. ostreatus*) and shown to have lytic activity against *P. tolaasii* (Sajben et al. 2011). However, none of the mentioned bacteriophages were investigated at commercial scale to

determine their efficacy. This is due in part to the complex and poorly understood taxonomy of the target pathogen. Theoretically, a combination of different bacteriophages (varying from one another in the host range and source of isolation) will make an aggressive biological control cocktail which is able to overcome the diversity of pathogenic pseudomonads in the mushroom compost and casing (Ramirez et al. 2018). However, bacteriophages are not similar to classic static chemicals nor do they have a fixed composition; hence, they should be produced under a continuous renewal and adaptation process (Merabishvili et al. 2018). Targeted collection of pseudomonads used for evaluation of bacteriophages needs to be renewed annually with the new isolates originating from mushroom farms where the cocktail will be used. A comprehensive guideline for selection of appropriate bacteriophages to be used in the process of bacteriophage cocktail design and production was described by Merabishvili et al. (2018).

Phage therapy can be considered either as a standalone control method or in combination with other biocontrol methods in mushroom production facilities. For instance, biological control of bacterial blotch with antagonistic *P. fluorescens* reduced crop losses by 30 to 60%, while treating the mushrooms with a mixture of the antagonist and bacteriophages reduced crop losses up to 80% (Guillaumes et al. 1998). However, while the combination of bacteriophages with other biocontrol methods is feasible, some important factors that affect the phage infectivity (i.e., compost/casing temperature, pH, heavy metals concentration, and conductivity) should be taken into account to prevent the inactivation of phage population (Svircev et al. 2011). Furthermore, timely application of phage mixture on the host bacterium is a pivotal determinant in the bactericidal effect of bacteriophages (Kim et al. 2011). Bactericidal effect decreases as the ratio of a host bacterium and its phage increases. Inoculation of phages until 4.5 h post incubation of *P. tolaasii* resulted in lysis of the host bacterium, while phage inoculation at 6 h resulted in incomplete bacterial lysis and maximal bacterial growth was obtained (Kim et al. 2011). Having some advantages, phage therapy can fail in the field due to either the limited range of pathogens affected by the phage or emergence of phage-resistant variants of the pathogen. Phage-resistant strains of *P. tolaasii* were isolated upon the successful application of phage therapy in mushroom farms (Park et al. 2016). Jones et al. (2007) explained ways to combat the development of resistance to phage treatment. Quality, safety, and efficacy requirements for applying phage therapy in agricultural commodities, clinical environments, and food systems are detailed by Pirnay et al. (2018), while the guidelines for bacteriophage product certification were described elsewhere (Fauconner 2018).

Conclusion and Retrospect

Research over the last 100 years has provided a foundation of knowledge about bacterial blotch of cultivated mushrooms that includes a broad understanding of the breadth of pathogens that cause blotch, and the biotic and abiotic factors that influence disease development. This groundwork and recent technological advancements ensure that in the coming years we will integrate our knowledge into a comprehensive understanding of the ecology of the blotch pathogens and disease development in the context of the complete compost/casing microbiome and mushroom production system. Additionally, these technologies will have impacts well beyond blotch control. For example, it is clear that the knowledge of the compost/casing microbiome will further our understanding of the “devome,” which is defined as the interaction of mushroom development with the mushroom microbiome including the microbiome of the compost/casing microbiome and vice versa.

Whole genome sequencing (WGS) will allow us to first identify sequences that can be used in loop-mediated isothermal amplification (LAMP) and other assays to detect and quantify various pathogen populations real-time in mushroom production houses. Second, WGS will allow us to predict and test predictions of which genes are involved in mushroom pathogenicity. This in turn will allow us to target these mechanisms for control just as tolaasin has been targeted in recent years (Tsukamoto et al. 1998, 2001). Third, WGS will allow us to determine whether the pathogens have

previously encountered predators (phage and *Bdellovibrio*) that have left signatures in their DNA. Knowledge of previous predation should help us to strategize ways to avoid resistance. Fourth, WGS of any of the organisms isolated will further our understanding of the individuals that make up the compost/casing microbiome and the interplay of the compost/casing microbiome and development in the mushroom (the devome). Lastly, the WGS of *A. bisporus* provides clues to its nutritional and developmental needs (Morin et al. 2012). There may be additional genes that could be mined as targets for disease and specifically blotch control as discussed above.

We now have the technology to explore the microbial dark matter consisting of organisms that are not easily culturable (Kertesz and Thai 2018). For much of the time since Tolaas, we were only able to study those microorganisms that we could grow in culture. Metagenomics, transcriptomics, and metabolomics are now being used to help us understand which organisms are present, what they are doing, and how they are doing it, respectively. We now know the dominant members of the casing microbiome (McGee et al. 2017a, b; Vieira and Pecchia 2017) and understand some of the changes that occur during composting. Coupling these tools to spatial and temporal analyses we should be able to predict specific interactions and their outcomes in both the compost/casing microbiome and devome.

‘Omics,’ specific detection and monitoring techniques, and additional technologies will allow us to answer many of the outstanding questions that need to be answered for blotch management including but not limited to:

- Are differences in coloration or development of blotch symptoms correlated to different species (e.g., *P. tolaasii* causing brown blotch vs. *P. agarici* causing yellow blotch)?
- What is the relative contribution of each potential blotch-causing species in the mushroom yield losses, and do various pathogens compete each other or inhibit one another?
- Are there differences in the threshold populations for each pathogen on the mushroom cap or casing layer under different microclimatic conditions to trigger blotch symptoms?
- Which epidemiological factors influence the incidence and dispersal of blotch pathogens?
- Do different pathogens respond differently to cap resistance to the blotch disease and does the importance of resistance change with proceeding in flush numbers?
- Are pathogens isolated during the first break less or more virulent than those isolated during the second or third break?
- Do all the mushroom-associated pseudomonad species respond in the same way to environmental conditions?
- Is there any relationship between the cap resistance to postharvest browning and resistance to bacterial blotch?

Control of bacterial blotch will be one of many end results of the application of these tools to mushroom production. However, the most promising area of research open to us because of the tools currently at our disposal may be the explanation of mushroom gene expressions via transcriptomics, exploration of the devome, and elucidation of the role of compost/casing microbiome in mushroom development. We already suspect that microorganisms drive nutrient availability by breaking down substrate, thus triggering development of pins. We will now explore the role of compost, casing, and mycelial associated microorganisms in driving development. This will provide the information we need to not only devise management strategies for blotch without compromising, promoting mushroom development, but should provide the information needed to increase yields through more complete use of mushroom substrate in later breaks.

Acknowledgments

Our thanks to Mozhdé Hamidzade (Department of Plant Protection, Shiraz University, Iran) for her assistance in the photography of blotch symptoms and

Justin Wheeler for his development of Figure 1. Special thanks to the Penn State Department of Plant Pathology and Environmental Microbiology, the College of Agricultural Sciences, and the Penn State Microbiome Center for their support.

Literature Cited

- Adams, M. H. 1959. Bacteriophages. Interscience Publishers, New York.
- Aslani, M. A., Harighi, B., and Abdollahzadeh, J. 2018. Screening of antifungal bacteria isolated from wild growing mushrooms as potential biological control agents against brown blotch and internal stipe necrosis diseases of *Agaricus bisporus*. *Biol. Control* 119:20-26.
- Ayers, T. T., and Lambert, E. B. 1955. Controlling mushroom diseases with chlorinated water. *Plant Dis. Rep.* 39:829-836.
- Beyer, D. M. 1993. Observation of Bacterial Blotch. *Mushroom News* 41:11-14.
- Beyer, D. M. 2005. Bacterial Blotch Part II. *Mushroom News* 53:14-20.
- Błaszczak, L., Siwulski, M., Sobieralski, K., and Frużyńska-Józwiak, D. 2013. Diversity of *Trichoderma* spp. causing *Pleurotus* green mould diseases in Central Europe. *Folia Microbiol. (Praha)* 58:325-333.
- Braun-Kiewnick, A., and Sands, D. C. 2001. *Pseudomonas*. Pages 84-120 in: Laboratory Guide for Identification of Plant Pathogenic Bacteria, 3rd Ed., N. D. Schaad, ed. APS Press, St Paul, MN.
- Brodey, C. L., Rainey, P. B., Tester, M., and Johnstone, K. 1991. Bacterial blotch disease of the cultivated mushroom is caused by an ion-channel forming lipopeptide toxin. *Mol. Plant-Microbe Interact.* 4:407-411.
- Bull, C. T., Clarke, C. R., Cai, R., Vinatzer, B. A., Jardini, T. M., and Koike, S. T. 2011. Multilocus sequence typing of *Pseudomonas syringae* sensu lato confirms previously described genomospecies and permits rapid identification of *P. syringae* pv. *coriandricola* and *P. syringae* pv. *apii* causing bacterial leaf spot on parsley. *Phytopathol.* 101:847-858.
- Bull, C. T., Duffy, B., Voisard, C., Défago, G., Keel, C., and Haas, D. 2001. Characterization of spontaneous *gacS* and *gacA* regulatory mutants in *Pseudomonas fluorescens* bicontrol strain CHA0. *Antonie van Leeuwenhoek* 79:327-336.
- Bull, C. T., and Koike, S. T. 2015. Practical benefits of knowing the enemy: Modern molecular tools for diagnosing the etiology of bacterial diseases and understanding the taxonomy and diversity of plant pathogenic bacteria. *Annu. Rev. Phytopathol.* 53:157-180.
- Carrasco, J., Tello, M. L., de Toro, M., Tkacz, A., Poole, P., Pérez-Clavijo, M., and Preston, G. 2019. Casing microbiome dynamics during button mushroom cultivation: implications for dry and wet bubble diseases. *Microbiology* 165: 611-624.
- Champion, A. B., Barrett, E. L., Palleroni, N. J., Soderberg, K. L., Kunisawa, R., Contopoulou, R., Wilson, A. C., and Doudoroff, M. 1980. Evolution in *Pseudomonas fluorescens*. *J. Gen. Microbiol.* 120:485-511.
- Cho, K. H., and Kim, Y. K. 2003. Two types of ion channel formation of tolaasin, a *Pseudomonas* peptide toxin. *FEMS Microbiol. Lett.* 221:221-226.
- Chowdhury, P. R., Pay, J., and Braithwaite, M. 2007. Isolation, identification and ecology of *Ewingella americana* (the causal agent of internal stipe necrosis) from cultivated mushrooms in New Zealand. *Australas. Plant Pathol.* 36:424.
- Cole, A. L. J., and Skellerup, M. V. 1986. Ultrastructure of the interaction of *Agaricus bisporus* and *Pseudomonas tolaasii*. *Trans. Br. Mycol. Soc.* 87: 314-316.
- Corbell, N., and Loper, J. E. 1995. A global regulator of secondary metabolite production in *Pseudomonas fluorescens* Pf-5. *J. Bacteriol.* 177:6230-6236.
- d'Herelle, F. 1931. Bacteriophage as a treatment in acute medical and surgical infections. *Bull. N. Y. Acad. Med.* 7:329-348.
- Dawoud, M. E. A., and Eweis, M. 2006. Phytochemical control of edible mushrooms pathogenic bacteria. *J. Food Agric. Environ.* 4:321-324.
- De Vos, P., Goor, M., Gillis, M., and De Ley, J. 1985. Ribosomal ribonucleic acid cistron similarities of phytopathogenic *Pseudomonas* species. *Int. J. Syst. Bacteriol.* 35:169-184.
- Eastwood, D., Green, J., Grogan, H., and Burton, K. 2015. Viral agents causing brown cap mushroom disease of *Agaricus bisporus*. *Appl. Environ. Microbiol.* 81:7125-7134.
- EPPO. 2010. Fungal diseases on cultivated mushrooms of *Agaricus* spp. EPPO Bull. 40:270-273.
- Fahy, P. C., and Bradley, J. K. 1987. Managing *Pseudomonas tolaasii*, Bacterial Blotch of *Agaricus* spp., by Integrated Biological and Chemical Practices. Pages 944-949 in: Plant Pathogenic Bacteria. Current Plant Science and Biotechnology in Agriculture. E. L. Civerolo, A. Collmer, R. E. Davis, and A. G. Gillaspie, eds. Vol. 4. Springer, Dordrecht, The Netherlands.
- Fahy, P. C., Nair, N. G., and Bradley, J. K. 1981. Epidemiology and biological control of bacterial blotch caused by *Pseudomonas tolaasii*. *Mushroom Sci.* 11:343-352.
- FAOSTAT. 2017. Food and Agriculture Organization of the United Nations, FAOSTAT database, available at <http://www.fao.org/faostat/en/>.
- Fauconnier, A. 2018. Guidelines for Bacteriophage Product Certification. Pages 253-268 in: Bacteriophage Therapy. Methods in Molecular Biology. J. Azeredo and S. Sillankorva, eds. Vol. 1693. Humana Press, New York.
- Fermor, T. R. 1987. Bacterial diseases of edible mushrooms and their control. *Dev. Crop Sci.* 10:361-370.
- Fermor, T. R., Henry, M. B., Fenlon, J. S., Glenister, M. J., Lincoln, S. P., and Lynch, J. M. 1991. Development and application of a biocontrol system for bacterial blotch of the cultivated mushroom. *Crop Prot.* 10:271-278.
- Fermor, T. R., and Lynch, J. M. 1988. Bacterial blotch disease of the cultivated mushroom *Agaricus bisporus*: screening, isolation and characterization of bacteria antagonistic to the pathogen (*Pseudomonas tolaasii*). *J. Appl. Bacteriol.* 65:179-187.
- Fett, W. F., Wells, J. M., Cescutti, P., and Wijey, C. 1995. Identification of exopolysaccharides produced by fluorescent pseudomonads associated with commercial mushroom (*Agaricus bisporus*) production. *Appl. Environ. Microbiol.* 61:513-517.
- Flegg, P. B., and Wood, D. A. 1985. Growth and fruiting. Pages 141-177 in: The Biology and Technology of the Cultivated Mushroom. P. B. Flegg, D. M. Spencer, and D. A. Wood, eds. Wiley, Chichester, UK.
- Fletcher, J. T. 1979. Bacteria and mushrooms. *Mushroom J.* 82:451-457.
- Fletcher, J. T., and Gaze, R. H. 2007. Mushroom Pest and Disease Control: A Colour Handbook. CRC Press, Boca Raton, FL.
- Fletcher, J. T., White, P. F., and Gaze, R. H. 1989. Mushrooms: Pest and disease control, 2nd ed. Intercept Limited, Andover, Hants, England.
- Fravel, D. R. 2005. Commercialization and implementation of biocontrol. *Annu. Rev. Phytopathol.* 43:337-359.
- Frey-Klett, P., Burlinson, P., Deveau, A., Barret, M., Tarkka, M., and Sarniguet, A. 2011. Bacterial-fungal interactions: hyphens between agricultural, clinical, environmental, and food microbiologists. *Microbiol. Mol. Biol. Rev.* 75: 583-609.
- Gagliardi, J. V., Angle, J. S., Germida, J. J., Wyndham, R. C., Chanway, C. P., Watson, R. J., Greer, C. W., McIntyre, T., Yu, H. H., Levin, M. A., Russek-Cohen, E., Rosolen, S., Nairn, J., Seib, A., Martin-Heller, T., and Wisse, G. 2001. Intact soil-core microcosms compared with multi-site field releases for pre-release testing of microbes in diverse soils and climates. *Can. J. Microbiol.* 47:237-252.
- Gandy, D. G. 1967. A technique for screening bacteria causing brown blotch of cultivated mushrooms. *Rep. Glasshouse Crops Res. Inst.* 1967:150-154.
- Geels, F. P. 1995. *Pseudomonas tolaasii* control by kasugamycin in cultivated mushrooms (*Agaricus bisporus*). *J. Appl. Bacteriol.* 79:38-42.
- Geels, F. P., and Schippers, B. 1983. Selection of antagonistic fluorescent *Pseudomonas* spp. and their root colonization and persistence following treatment of seed potatoes. *Phytopathologische Zeitschrift* 108:193-206.
- Geels, F. P., van Griensven, L. J. L. D., and Rutjens, A. J. 1991. Chlorine dioxide and the control of bacterial blotch on mushrooms, caused by *Pseudomonas tolaasii*. Pages 437-442 in: Science and Cultivation of Edible Fungi. M. J. Maher, ed. Balkema, Rotterdam, The Netherlands.
- Ghequire, M. G. K., and De Mot, R. 2014. Ribosomally encoded antibacterial proteins and peptides from *Pseudomonas*. *FEMS Microbiol. Rev.* 38:523-568.
- Ghequire, M. G. K., Rokni-Zadeh, H., Zarrineh, P., and De Mot, R. 2013. Draft genome sequence of *Pseudomonas fluorescens* LMG 5329, a white line-inducing principle-producing bioindicator for the mushroom pathogen *Pseudomonas tolaasii*. *Genome Announc.* 1:e00383-13.
- Gill, W. M., and Cole, A. L. J. 1992. Cavity disease of *Agaricus bitorquis* caused by *Pseudomonas cepacia*. *Can. J. Microbiol.* 38:394-397.
- Gill, W. M., and Tsuneda, A. 1997. The interaction of the soft rot bacterium *Pseudomonas gladioli* pv. *agaricicola* with Japanese cultivated mushrooms. *Can. J. Microbiol.* 43:639-648.
- Godfrey, S. A., Harrow, S. A., Marshall, J. W., and Klena, J. D. 2001. Characterization by 16S rRNA sequence analysis of pseudomonads causing blotch disease of cultivated *Agaricus bisporus*. *Appl. Environ. Microbiol.* 67:4316-4323.
- Goor, M., Vantomme, R., Swings, J., Gillis, M., Kersters, K., and de Ley, J. 1986. Phenotypic and genotypic diversity of *Pseudomonas tolaasii* and white line reacting organisms isolated from cultivated mushrooms. *J. Gen. Microbiol.* 132:2249-2264.
- Grewal, P. S. 1991. Effects of *Caenorhabditis elegans* (Nematoda: Rhabditidae) on the spread of the bacterium *Pseudomonas tolaasii* in mushrooms (*Agaricus bisporus*). *Ann. Appl. Biol.* 118:47-55.
- Grewal, S. I., Han, B., and Johnstone, K. 1995. Identification and characterization of a locus which regulates multiple functions in *Pseudomonas tolaasii*, the cause of brown blotch disease of *Agaricus bisporus*. *J. Bacteriol.* 177:4658-4668.
- Grewal, S. I., and Rainey, P. B. 1991. Phenotypic variation of *Pseudomonas putida* and *P. tolaasii* affects the chemotactic response to *Agaricus bisporus* mycelial exudate. *J. Gen. Microbiol.* 137:2761-2768.
- Grogan, H. M. 2006. Fungicide control of mushroom cobweb disease caused by *Cladobotryum* strains with different benzimidazole resistance profiles. *Pest Manag. Sci.* 62:153-161.
- Guillaumes, J., Houdeau, G., Germain, R., and Oliver, J. M. 1998. Amélioration de la lutte biologique contre *Pseudomonas tolaasii* utilisation de bacteriophages. *EPPO Bull.* 18:77-82.
- Henkels, M. D., Kidarsa, T. A., Shaffer, B. T., Goebel, N. C., Burlinson, P., Mavrodi, D. V., Bentley, M. A., Rangel, L. I., Davis, E. W., Thomashow, L. S., Zabriskie, T. M., Preston, G. M., and Loper, J. E. 2014. *Pseudomonas protegens* Pf-5 causes discoloration and pitting of mushroom caps due to the production of antifungal metabolites. *Mol. Plant-Microbe Interact.* 27: 733-746.
- Henry, M. B., Lynch, J. M., and Fermor, T. R. 1991. Role of siderophores in the biocontrol of *Pseudomonas tolaasii* by fluorescent pseudomonad antagonists. *J. Appl. Bacteriol.* 70:104-108.
- Hesse, C., Schulz, F., Bull, C. T., Shaffer, B. T., Yan, Q., Shapiro, N., Hassan, K. A., Varghese, N., Elbourne, L. D. H., Paulsen, I. T., Kyrpides, N.,

Ebrahim Osdaghi

Dr. Osdaghi received his B.S. in plant protection and M.S. in plant pathology from the University of Tabriz and Tarbiat Modares University in 2007 and 2009, respectively, in Iran. Since 2009 to 2014, he served as a lecturer at Ramin University (Iran), where he was responsible for teaching general plant pathology, microbiology, and plant bacteriology courses. He attended to Shiraz University (Iran) in 2014, and received his Ph.D. in plant pathology in 2017. During the Ph.D. program, he specialized in the population genetics and phylogeography of seed-borne quarantine bacterial plant pathogens. Currently, as a research associate, he is running a joint project on the bacterial blotch of edible mushrooms at Shiraz University in collaboration with Pennsylvania State University members.

Samuel J. Martins

Dr. Samuel J. Martins is a postdoctoral researcher and lab manager in the Department of Plant Pathology and Environmental Microbiology at Penn State University. He received his B.S. in agronomy from the Federal University of Lavras (Lavras, Brazil), as well as an M.S. and Ph.D. in plant pathology from the same institution, with part of his Ph.D. completed at the University of Delaware. From 2015 to 2017, he served as an assistant professor at the Federal University of Goiás (Goiânia, Brazil), where he taught IPM and general plant pathology. His research focuses on manipulating and extracting bacteria from the phytobiome to manage plant disease, improve nutrient uptake and diminish the impact of abiotic stress, such as drought, by increasing water use efficiency. He also works with translational taxonomy using multilocus sequence analysis (MLSA) and whole genome to distinguish among closely related bacterial plant pathogens. At Penn State, he is engaged in research involving blotch on mushrooms (*Agaricus bisporus*) with the ultimate goal of providing the industry with biologically based disease management tactics to add to the environmental management practices already in place.

Laura Ramos-Sepulveda

Dr. Ramos-Sepulveda earned her B.A. in horticulture from the University of Puerto Rico at Mayaguez and received her Ph.D. in plant pathology from The Pennsylvania State University. Currently, she is an assistant professor at Millersville University of Pennsylvania. Her teaching specializations include general microbiology, mycology, and virology. Her research focuses on bacterial diseases of edible mushrooms with the goal of developing reliable control methods against bacterial diseases. More specifically, she is interested in studying the bacteria-fungi interactions, the epidemiology of the disease, and the disease after harvest, and continues to work in collaboration with Penn State University.

Fabrício Rocha Vieira

Dr. Fabrício Vieira is a postdoctoral fellow in the Plant Pathology and Environmental Microbiology Department at Pennsylvania State University. Vieira received a B.S. in agronomy engineering from the Superior School of Agronomy (São Paulo, Brazil), and an M.S. and Ph.D. in agronomy from Sao State University (São Paulo, Brazil). He also has specializations as a visiting scholar on mushroom research programs at Fujian Agriculture and Forestry University (China), Tokyo University of Agriculture and Technology (Japan), and Wageningen University (The Netherlands). His career and research are fully dedicated to study mushroom cultivation systems and cover all aspects of the microbiology associated with these systems (mainly for *Agaricus bisporus* and *Pleurotus ostreatus* mushroom species). Vieira has specialized in the study of microbial communities associated with mushroom cultivation by using molecular biology techniques such as amplicon- and genome-sequencing as well as bioinformatics. The main focus of his research is to understand how to manipulate these microorganisms efficiently during a mushroom cropping process targeting disease biocontrol (especially green mold, *Trichoderma* spp.), plant biomass carbohydrate conversion, and fungi-bacteria-fungi interactions during mushroom development.

John A. Pecchia

Dr. John Pecchia is an associate research professor of mushrooms and director of the Mushroom Research Center and Mushroom Spawn Laboratory in the Department of Plant Pathology and Environmental Microbiology at Pennsylvania State University. Dr. Pecchia received a B.S. in environmental biology from Lock Haven University, an M.S. in biology from Bloomsburg University, and a Ph.D. in plant pathology at Penn State University, where he studied air emissions generated from Phase I mushroom composting. Following completion of his Ph.D., Dr. Pecchia worked as a postdoc at The Ohio State University studying on-farm composting and worked at several smaller colleges teaching biology and environmental science classes. Dr. Pecchia's lab group currently focuses on increasing production efficiency of mushroom production by studying ways to improve disease control and by investigating the role the substrate microbiome plays in disease suppression, fruit body formation, and nutrient availability.

David Meigs Beyer

Dr. David Meigs Beyer is a professor in the Department of Plant Pathology and Environmental Microbiology at Pennsylvania State University. He received a B.S. in biology from Syracuse University in 1977 and an M.S. in plant pathology at Pennsylvania State University in 1979. He then worked at the largest commercial mushroom farm in Canada for 12 years. He earned a Ph.D. in plant pathology at Pennsylvania State University in 1996 and has been on the faculty ever since. His primary responsibility is the extension program for the North American mushroom industry. He conducts workshops, conferences, and farm visits for mushroom growers. His applied research program covers all aspects of mushroom cultivation, including composting, mushroom nutrition, compost and casing alternatives, fresh mushroom quality, and mushroom disease biology and control.

Terrence H. Bell

Dr. Terrence Bell is an assistant professor of phytobiomes in the Department of Plant Pathology and Environmental Microbiology at Pennsylvania State University. Originally from Canada, Terrence received a B.S. in biology from Queen's University, an M.S. in biology from the University of Western Ontario (2008), and his Ph.D. in natural resource sciences from McGill University in Montreal (2013), where he studied microorganisms from contaminated soils in the high arctic. This was followed by a postdoc at l'Université de Montréal, which focused on the interactions between willows and microorganisms in contaminated soils. During a postdoctoral fellowship at Cornell, and now in his own lab, he works primarily on microorganisms in agricultural systems. Primary objectives in Dr. Bell's group are to understand the controls on microbial influx to managed and natural systems, and the constraints on microbial survival in diverse environments.

Yinong Yang

Yinong Yang is a professor in the Department of Plant Pathology and Environmental Microbiology and the Huck Institutes of Life Sciences at the Pennsylvania State University. He received a B.S. in biology from Hangzhou University in 1982, an M.S. in botany from the University of South Florida in 1990, and a Ph.D. in plant molecular and cellular biology from the University of Florida in 1994. After a postdoctoral position at the Waksman Institute at Rutgers University, he became an assistant and associate professor at the University of Arkansas. He joined the faculty at Penn State in 2006 and has been working in the areas of molecular plant-microbe interactions and genome engineering. His recent studies focus on improving the CRISPR/Cas-mediated genome editing technology for its broad applications in precision breeding and disease management of agricultural crops.

Kevin L. Hockett

Kevin Hockett is an assistant professor of microbial ecology and a Lloyd Huck Early Career Professor in the Department of Plant Pathology and Environmental Microbiology at the Pennsylvania State University. Hockett earned his B.S. in microbiology from Oregon State University in 2005, his Ph.D. from the University of California, Berkeley, in 2012, and trained as a postdoc at the University of Arizona from 2012 to 2016. His group focuses on intermicrobial competition among plant and mushroom associated bacteria. He is particularly interested in a diverse group of narrow spectrum protein toxins known as bacteriocins. These toxins are encoded by many different bacteria, often with a single strain encoding multiple bacteriocins. More broadly, his group is interested in the ecology governing microbial interactions, and how knowledge of this ecology can be leveraged to suppress plant and mushroom diseases.

Carolee T. Bull

Dr. Carolee Bull is a professor of plant pathology and bacterial systematics and serves as the department head for the Department of Plant Pathology and Environmental Microbiology at Pennsylvania State University. Bull also serves as the director of the Penn State Microbiome Center. Research in the Bull lab focuses on translational bacterial taxonomy to elucidate the etiology of bacterial pathogens of plants and mushrooms and use this knowledge to develop alternatives to chemicals for control of plant pathogens. Bull is the project director for an SCRI project using multiple approaches including diagnostic metagenomics to develop management strategies for seedborne diseases caused by *Pseudomonas syringae* on cucurbit, beet, and chard food and seed crops. Dr. Bull serves as the convener of the Committee on the Taxonomy of Plant Pathogenic Bacteria for the International Society of Plant Pathology and as the secretary of the Judicial Commission of the International Committee for the Systematics of Prokaryotes. She provides virtual and live bacterial taxonomy and mentorship, lectures, and workshops for classes, universities, and scientific societies worldwide. Bull received the highest award for service to the U.S. in agriculture for her mentorship of underrepresented students in agricultural science.

- Woyke, T., and Loper, J. E. 2018. Genome-based evolutionary history of *Pseudomonas* spp. *Environ. Microbiol.* 20:2142-2159.
- Hwang, M. S. H., Morgan, R. L., Sarkar, S. F., Wang, P. W., and Guttman, D. S. 2005. Phylogenetic characterization of virulence and resistance phenotypes of *Pseudomonas syringae*. *Appl. Environ. Microbiol.* 71:5182-5191.
- Iacobellis, N. S., and Lo Cantore, P. 2003. *Pseudomonas 'reactans'* a new pathogen of cultivated mushrooms. Pages 595-605 in: *Pseudomonas syringae Pathovars and Related Pathogens*. N. S. Iacobellis, A. Collmer, S. W. Hutcheson, J. W. Mansfield, C. E. Morris, J. Muriello, N. W. Schaad, D.E. Stead, G. Surico, and M. S. Ullrich, eds. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Jones, J. B., Jackson, L. E., Balogh, B., Obradovic, A., Iriarte, F. B., and Momol, M. T. 2007. Bacteriophages for plant disease control. *Annu. Rev. Phytopathol.* 45:245-262.
- Kabel, M. A., Jurak, E., Mäkelä, M. R., and de Vries, R. P. 2017. Occurrence and function of enzymes for lignocellulose degradation in commercial *Agaricus bisporus* cultivation. *Appl. Microbiol. Biotechnol.* 101:4363-4369.
- Kertesz, M. A., and Thai, M. 2018. Compost bacteria and fungi that influence growth and development of *Agaricus bisporus* and other commercial mushrooms. *Appl. Microbiol. Biotechnol.* 102:1639-1650.
- Kim, M. H., Park, S. W., and Kim, Y. K. 2011. Bacteriophages of *Pseudomonas tolaasii* for the biological control of brown blotch disease. *J. Korean Soc. Appl. Biol. Chem.* 54:99-104.
- King, E. O., Ward, M. K., and Raney, E. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. *J. Lab. Clin. Med.* 44:301.
- Kleuskens, A. J. J. 1983. Research on the relationship between the drying time, climate and irrigation and the occurrence of bacterial spots. *Champignoncultuur* 27:301-305.
- Lamichhane, J. R., Osdaghi, E., Behlau, F., Köhl, J., Jones, J. B., and Aubertot, J. N. 2018. Thirteen decades of anti-microbial copper compounds applied in agriculture. A review. *Agron. Sustain. Dev.* 38:28.
- Largeteau, M. L., and Savoie, J. M. 2010. Microbially induced diseases of *Agaricus bisporus*: biochemical mechanisms and impact on commercial mushroom production. *Appl. Microbiol. Biotechnol.* 86:63-73.
- Laville, J., Voisard, C., Keel, C., Maurhofer, M., Defago, G., and Haas, D. 1992. Global control in *Pseudomonas fluorescens* mediating antibiotic synthesis and suppression of black root rot of tobacco. *Proc. Natl. Acad. Sci.* 89:1562-1566.
- Lee, H.-I., and Cha, J.-S. 1998. Cloning of a DNA fragment specific to *Pseudomonas tolaasii* causing bacterial brown blotch disease of oyster mushroom (*Pleurotus ostreatus*). *Korean J. Plant Pathol.* 14:177-183.
- Lee, H. I., Jeong, K. S., and Cha, J. S. 2002. PCR assays for specific and sensitive detection of *Pseudomonas tolaasii*, the cause of brown blotch disease of mushrooms. *Lett. Appl. Microbiol.* 35:276-280.
- Lelliott, R. A., Billing, E., and Hayward, A. C. 1966. A determinative scheme for the fluorescent plant pathogenic pseudomonads. *J. Appl. Bacteriol.* 29:470-489.
- Liao, Y.-M., Tu, C.-C., and Jeng, J.-J. 1980. Control of bacterial blotch of mushroom. *Taiwan Mushrooms* 4:34-41.
- Lincoln, S. P., Fermor, T. R., Stead, D. E., and Sellwood, J. E. 1991. Bacterial soft-rot of *Agaricus bitorquis*. *Plant Pathol.* 40:136-144.
- Lomax, K. M. 2007. Dew point temperature related to wet mushroom caps. *Mushroom News* 55:4-10.
- Lynch, T. 2018. *Mushroom Cultivation: An Illustrated Guide to Growing Your Own Mushrooms at Home*. Quarry Books, Beverly, MA.
- Markowitz, N. L., and Bodine, D. 1989. Studies on the use of stabilized chlorine dioxide (Oxysan) for improving mushroom quality and shelf life. *Mushroom Trade Rep.* 8:5-7.
- Matsuzaki, S., Rashel, M., Uchiyama, J., Sakurai, S., Ujihara, T., Kuroda, M., Ikeuchi, M., Tani, T., Fujieda, M., Wakiguchi, H., and Imai, S. 2005. Bacteriophage therapy: a revitalized therapy against bacterial infectious diseases. *J. Infect. Chemother.* 11:211-219.
- McGee, C. F., Byrne, H., Irvine, A., and Wilson, J. 2017a. Diversity and dynamics of the DNA and cDNA-derived bacterial compost communities throughout the *Agaricus bisporus* mushroom cropping process. *Ann. Microbiol.* 67:751-761.
- McGee, C. F., Byrne, H., Irvine, A., and Wilson, J. 2017b. Diversity and dynamics of the DNA and cDNA-derived compost fungal communities throughout the commercial cultivation process for *Agaricus bisporus*. *Mycologia* 109:475-484.
- Mehnaz, S., Saleem, R. S., Yameen, B., Pianet, I., Schnakenburg, G., Pietraszkiewicz, H., Valeriotte, F., Josten, M., Sahli, H. G., Franzblau, S. G., and Gross, H. 2013. Lahorenoic acids A-C, ortho-dialkyl-substituted aromatic acids from the biocontrol strain *Pseudomonas aurantiaca* PB-St2. *J. Nat. Prod.* 76:135-141.
- Merabishvili, M., Pirnay, J. P., and De Vos, D. 2018. Guidelines to Compose an Ideal Bacteriophage Cocktail. Pages 99-110 in: *Bacteriophage Therapy. Methods in Molecular Biology*. J. Azeredo and S. Sillankorva, eds. Vol. 1693. Humana Press, New York.
- Miller, F. C., and Spear, M. 1995. Very large scale commercial trial of a biological control for mushroom blotch disease. *Mushroom Sci.* 14:635-642.
- Moore, E. R. B., Tindall, B. J., Martins Dos Santos, V. A. P., Pieper, D. H., Ramos, J. L., and Palleroni, N. J. 2006. Nonmedical: *Pseudomonas*. Pages 646-703 in: *The Prokaryotes*. M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer, and E. Stackebrandt, eds. Springer, New York.
- Moquet, F., Desmerger, C., Mamoun, M., Ramos-Guedes-Lafargue, M., and Olivier, J. M. 1999. A quantitative trait locus of *Agaricus bisporus* resistance to *Pseudomonas tolaasii* is closely linked to natural cap color. *Fungal Genet. Biol.* 28:34-42.
- Morin, E., Kohler, A., Baker, A. R., Foulongne-Oriol, M., Lombard, V., Nagy, L. G., Ohm, R. A., Patyshakuliyeva, A., Brun, A., Aerts, A. L., Bailey, A. M., Billette, C., Coutinho, P. M., Deakin, G., Doddapaneni, H., Floudas, D., Grimwood, J., Hildén, K., Kües, U., LaButti, K. M., Lapidus, A., Lindquist, E. A., Lucas, S. M., Murat, C., Riley, R. W., Salamov, A. A., Schmutz, J., Subramanian, V., Wösten, H. A., Xu, J., Eastwood, D. C., Foster, G. D., Sonnenberg, A. S. M., Cullen, D., de Vries, R. P., Lundell, T., Hibbett, D. S., Henrissat, B., Burton, K. S., Kerrigan, R. W., Challen, M. P., Grigoriev, I. V., and Martin, F. 2012. Genome sequence of the button mushroom *Agaricus bisporus* reveals mechanisms governing adaptation to a humic-rich ecological niche. *PNAS* 109:17501-17506.
- Mortishire-Smith, R. J., Nutkins, J. C., Packman, L. C., Brodey, C. L., Rainey, P. B., Johnstone, K., and Williams, D. H. 1991. Determination of the structure of an extracellular peptide produced by the mushroom saprotroph *Pseudomonas reactans*. *Tetrahedron* 47:3645-3654.
- Mu, L. L., Yun, Y. B., Park, S. J., Cha, J. S., and Kim, Y. K. 2015. Various pathogenic *Pseudomonas* strains that cause brown blotch disease in cultivated mushroom. *J. Appl. Biol. Chem.* 58:349-354.
- Mulet, M., Lalucat, J., and García-Valdés, E. 2010. DNA sequence-based analysis of the *Pseudomonas* species. *Environ. Microbiol.* 12:1513-1530.
- Munjal, A., Khanna, P. K., and Garcha, H. S. 1989. *In vitro* chemical and biological control of bacterial blotch of *Agaricus bisporus* (Sing.) Lange. *Mushroom Sci.* 12:667-677.
- Munsch, P., and Alatosava, T. 2002. The white-line-in agar test is not specific for the two cultivated mushroom associated pseudomonads, *Pseudomonas tolaasii* and *Pseudomonas 'reactans'*. *Microbiol. Res.* 157:7-11.
- Munsch, P., Alatosava, T., Martinen, N., Meyer, J. M., Christen, R., and Gardan, L. 2002a. *Pseudomonas costantinii* sp. nov., another causal agent of brown blotch disease, isolated from cultivated mushroom sporophores in Finland. *Int. J. Syst. Evol. Microbiol.* 52:1973-1983.
- Munsch, P., Geoffroy, V. A., Alatosava, T., and Meyer, J.-M. 2000. Application of Siderotyping for Characterization of *Pseudomonas tolaasii* and '*Pseudomonas reactans*' Isolates Associated with Brown Blotch Disease of Cultivated Mushrooms. *Appl. Environ. Microbiol.* 66:4834-4841.
- Munsch, P., Johnstone, K., and Alatosava, T. 2002b. Evidence for genotypic differences between the two siderovars of *Pseudomonas tolaasii*, cause of brown blotch disease of the cultivated mushroom *Agaricus bisporus*. *Microbiol. Res.* 157:93-102.
- Munsch, P., and Olivier, J. M. 1995. Biocontrol of bacterial blotch of the cultivated mushroom with lytic phages: some practical considerations. *Mushroom Sci.* 14: 595-602.
- Munsch, P., Olivier, J. M., and Houdeau, G. 1991. Experimental control of bacterial blotch by bacteriophages. Pages 389-396 in: *Science and Cultivation of Edible Fungi*. M. J. Maher, ed. Balkema, Rotterdam, The Netherlands.
- Murata, H., and Magae, Y. 1996. Toxin production in a mushroom pathogenic bacterium, *Pseudomonas tolaasii* strain PT814 is activated by signals present in a host, *Pleurotus ostreatus*, and those accumulating in the medium in the course of bacterial growth. Pages 483-494 in: *Mushroom biology and mushroom products*. D. J. Royse, ed. Pennsylvania State Univ. Press, College Park, PA.
- Nair, N. G., and Bradley, J. K. 1980. Mushroom blotch bacterium during cultivation. *Mushroom J.* 90:201-203.
- Nair, N. G., and Fahy, P. C. 1972. Bacteria Antagonistic to *Pseudomonas tolaasii* and their Control of Brown Blotch of the Cultivated Mushroom *Agaricus bisporus*. *J. Appl. Bacteriol.* 35:439-442.
- Nair, N. G., and Fahy, P. C. 1973. Toxin production by *Pseudomonas tolaasii* Paine. *Aust. J. Bio. Sci.* 26:509-512.
- Nair, N. G., and Fahy, P. C. 1976. Commercial application of biological control of mushroom bacterial blotch. *Aust. J. Agric. Res.* 27:415-422.
- Namazi, Z., Hasanazadeh, N., and Razmi, J. 2016. *Kocuria* sp. a potential antagonist of brown blotch caused by *Pseudomonas tolaasii*. *J. Biodivers. Environ. Sci.* 8: 159-166.
- Navarro, M. J., Gea, F. J., and González, A. J. 2018. Identification, incidence and control of bacterial blotch disease in mushroom crops by management of environmental conditions. *Sci. Hortic. (Amsterdam)* 229:10-18.
- Negus, D., Moore, C., Baker, M., Raghunathan, D., Tyson, J., and Sockett, R. E. 2017. Predator versus pathogen: How does predatory *Bdellovibrio bacteriovorus* interface with the challenges of killing Gram-negative pathogens in a host setting? *Annu. Rev. Microbiol.* 71:441-457.
- Nguyen, H. T. D., Yoon, S., Kim, M.-H., Kim, Y.-K., Yoon, M.-Y., Cho, Y.-H., Lim, Y., Shin, S. H., and Kim, D.-E. 2012. Characterization of bacteriophage φPto-bp6g, a novel phage that lyses *Pseudomonas tolaasii* causing brown blotch disease in mushrooms. *J. Microbiol. Methods* 91:514-519.
- Noble, R., Fermor, T. R., Lincoln, S., Dobrovin-Pennington, A., Evered, C., Mead, A., and Li, R. 2003. Primordia initiation of mushroom (*Agaricus bisporus*) strains on axenic casing materials. *Mycologia* 95:620-629.
- Nutkins, J. C., Mortishire-Smith, R. J., Packman, L. C., Brodey, C. L., Rainey, P. B., Johnstone, K., and Williams, D. H. 1991. Structure determination of tolaasin, an extracellular lipopeptide produced by the mushroom pathogen, *Pseudomonas tolaasii* Paine. *J. Am. Chem. Soc.* 113:2621-2627.
- Olivier, J. M., Guillau, J. S., and Martin, D. 1978. Study of a bacterial disease of mushroom caps. Pages 903-916 in: *Proceedings of the 4th International Conference on Plant Pathogenic Bacteria*, INRA, Angers, France.

- Olivier, J. M., and Guillaumes, J. 1981. Essais de lutte biologique contre la tache bactérienne. *Mushroom Sci.* 11:353-368.
- Olivier, J.-M., Mamoun, M., and Munsch, P. 1997. Standardization of a method to assess mushroom blotch resistance in cultivated and wild *Agaricus bisporus* strains. *Can. J. Plant Pathol.* 19:36-42.
- Paine, S. G. 1919. Studies in bacteriosis: II a brown blotch disease of cultivated mushrooms. *Ann. Appl. Biol.* 5:206-219.
- Palleroni, N. J. 1984. Gram-negative aerobic rods and cocci, family I: *Pseudomonadaceae*. Pages 141-199 in: *Bergey's Manual of Systematic Bacteriology*. N. R. Krieg and J. G. Holt, eds. Vol. 1. Williams and Wilkins, Baltimore, MD.
- Palleroni, N. J. 1993. *Pseudomonas* classification. A new case history in the taxonomy of Gram-negative bacteria. *Antonie van Leeuwenhoek* 64:231-251.
- Palleroni, N. J., Ballard, R. W., Ralston, E., and Doudoroff, M. 1972. Deoxyribonucleic acid homologies among some *Pseudomonas* species. *J. Bacteriol.* 110:1-11.
- Panke-Buisse, K., Poole, A. C., Goodrich, J. K., Ley, R. E., and Kao-Kniffin, J. 2015. Selection on soil microbiomes reveals reproducible impacts on plant function. *ISME J.* 9:980-989.
- Park, S. J., Han, J. H., and Kim, Y. K. 2016. Isolation of bacteriophage-resistant *Pseudomonas tolaasii* strains and their pathogenic characters. *J. Appl. Biol. Chem.* 59:351-356.
- Parret, A. H. A., Temmerman, K., and De Mot, R. 2005. Novel lectin-like bacteriocins of biocontrol strain *Pseudomonas fluorescens* Pf-5. *Appl. Environ. Microbiol.* 71:5197-5207.
- Partanen, P., Hultman, J., Paulin, L., Auvinen, P., and Romantschuk, M. 2010. Bacterial diversity at different stages of the composting process. *BMC Microbiol.* 10:94.
- Pecchia, J. A., Cortese, R., and Albert, I. 2014. Investigation into the microbial community changes that occur in the casing layer during cropping of the white button mushroom, *Agaricus bisporus*. Pages 309-313 in: *Proceedings of the 8th International Conference on Mushroom Biology and Mushroom Products*, New Delhi, M. Singh, ed.
- Pimay, J. P., Merabishvili, M., Van Raemdonck, H., De Vos, D., and Verbeke, G. 2018. Bacteriophage Production in Compliance with Regulatory Requirements. Pages 233-252 in: *Bacteriophage Therapy. Methods in Molecular Biology*. J. Azeredo and S. Sillankorva, eds. Vol. 1693. Humana Press, New York.
- Qiu, W., Huang, Y., Zhao, C., Lin, Z., Lin, W., and Wang, Z. 2019. Microflora of fresh white button mushrooms (*Agaricus bisporus*) during cold storage revealed by high-throughput sequencing and MALDI-TOF mass spectrometry fingerprinting. *J. Sci. Food Agric.* 99:4498-4503.
- Raaijmakers, J. M., De Bruijn, I., Nybroe, O., and Ongena, M. 2010. Natural functions of lipopeptides from *Bacillus* and *Pseudomonas*: More than surfactants and antibiotics. *FEMS Microbiol. Rev.* 34:1037-1062.
- Rainey, P. B. 1991a. Effect of *Pseudomonas putida* on hyphal growth of *Agaricus bisporus*. *Mycol. Res.* 95:699-704.
- Rainey, P. B. 1991b. Phenotypic variation of *Pseudomonas putida* and *P. tolaasii* affects attachment to *Agaricus bisporus* mycelium. *J. Gen. Microbiol.* 137: 2769-2779.
- Rainey, P. B., Brodey, C. L., and Johnstone, K. 1991. Biological properties and spectrum of activity of tolaasin, a lipodepsipeptide toxin produced by the mushroom pathogen *Pseudomonas tolaasii*. *Physiol. Mol. Plant Pathol.* 39: 57-70.
- Rainey, P. B., Brodey, C. L., and Johnstone, K. 1992. Biology of *Pseudomonas tolaasii*, cause of brown blotch disease of the cultivated mushroom. *Adv. Plant Pathol.* 8:95-117.
- Rainey, P. B., Brodey, C. L., and Johnstone, K. 1993. Identification of a gene cluster encoding three high-molecular-weight proteins, which is required for synthesis of tolaasin by the mushroom pathogen *Pseudomonas tolaasii*. *Mol. Microbiol.* 8:643-652.
- Rainey, P. B., Cole, A. L. J., Farmor, T. R., and Wood, D. A. 1990. A model system for examining involvement of bacteria in basidiome initiation of *Agaricus bisporus*. *Mycol. Res.* 94:191-195.
- Ramette, A., Frapolli, M., Fischer-Le Saux, M., Gruffaz, C., Meyer, J. M., Défago, G., Sutra, L., and Moëne-Loccoz, Y. 2011. *Pseudomonas protegens* sp. nov., widespread plant-protecting bacteria producing the biocontrol compounds 2,4-diacetylphloroglucinol and pyoluteorin. *Syst. Appl. Microbiol.* 34:180-188.
- Ramirez, K., Cazarez-Montoya, C., Lopez-Moreno, H. S., and Castro-del Campo, N. 2018. Bacteriophage cocktail for biocontrol of *Escherichia coli* O157:H7: Stability and potential allergenicity study. *PLoS One* 13:e0195023.
- Reder-Christ, K., Schmidt, Y., Dörr, M., Sahl, H.-G., Josten, M., Raaijmakers, J. M., Gross, H., and Bendas, G. 2012. Model membrane studies for characterization of different antibiotic activities of lipopeptides from *Pseudomonas*. *Biochim. Biophys. Acta* 1818:566-573.
- Reyes, J. E., Venturini, M. E., Oria, R., and Blanco, D. 2004. Prevalence of *Ewingella americana* in retail fresh cultivated mushrooms (*Agaricus bisporus*, *Lentinula edodes* and *Pleurotus ostreatus*) in Zaragoza (Spain). *FEMS Microbiol. Ecol.* 47:291-296.
- Riley, M. A., and Wertz, J. E. 2002. Bacteriocins: Evolution, ecology, and application. *Annu. Rev. Microbiol.* 56:117-137.
- Rokni-Zadeh, H., Li, W., Sanchez-Rodriguez, A., Sinnaeve, D., Rozenski, J., Martins, J. C., and De Mot, R. 2012. Genetic and functional characterization of cyclic lipopeptide white-line-inducing principle (WLIP) production by rice rhizosphere isolate *Pseudomonas putida* RW10S2. *Appl. Environ. Microbiol.* 78:4826-4834.
- Rokni-Zadeh, H., Li, W., Yilma, E., Sanchez-Rodriguez, A., and De Mot, R. 2013. Distinct lipopeptide production systems for WLIP (white line-inducing principle) in *Pseudomonas fluorescens* and *Pseudomonas putida*. *Environ. Microbiol. Rep.* 5:160-169.
- Rokni-Zadeh, H., Mangas-Losada, A., and De Mot, R. 2011. PCR detection of novel non-ribosomal peptide synthetase genes in lipopeptide-producing *Pseudomonas*. *Microb. Ecol.* 62:941-947.
- Royse, D. J., and Wuest, P. J. 1980. Mushroom brown blotch: effects of chlorinated water on disease intensity and bacterial populations in casing soil and on pilei. *Phytopathology* 70:902-905.
- Russo, A., Filippi, C., Tombolini, R., Toffanin, A., Bedini, S., Agnolucci, M., and Nuti, M. 2003. Interaction between gfp-tagged *Pseudomonas tolaasii* P12 and *Pleurotus eryngii*. *Microbiol. Res.* 158:265-270.
- Sajben, E., Manczinger, L., Nagy, A., Kredics, L., and Vágvolgyi, C. 2011. Characterization of pseudomonads isolated from decaying sporocarps of oyster mushroom. *Microbiol. Res.* 166:255-267.
- Sajben-Nagy, E., Maróti, G., Kredics, L., Horváth, B., Párducz, A., Vágvolgyi, C., and Manczinger, L. 2012. Isolation of new *Pseudomonas tolaasii* bacteriophages and genomic investigation of the lytic phage BF7. *FEMS Microbiol.* 332:162-169.
- Samson, R., Houdeau, G., Kharma, P., Guillaumes, J., and Olivier, J. M. 1987. Variability of fluorescent pseudomonas population in composts and casing soils used from mushroom cultures. Pages 19-25 in: *Proceedings of the International Symposium on Scientific and Technical Aspects of Cultivating Edible Fungi*. P. J. Wuest, D. J. Royse, and R. B. Beelman, eds. Elsevier Science Publishers, Amsterdam, The Netherlands.
- Savoie, J.-M., Mata, G., and Largeteau, M. 2016. New prospects in pathogen control of button mushroom cultures. Pages 93-110 in: *Mushroom Biotechnology Developments and Applications*. M. Petre, ed. Academic Press, London.
- Saxon, E. B., Jackson, R. W., Bhumbra, S., Smith, T., and Sockett, R. E. 2014. *Bdellovibrio bacteriovorus* HD100 guards against *Pseudomonas tolaasii* brown-blotch lesions on the surface of post-harvest *Agaricus bisporus* supermarket mushrooms. *BMC Microbiol.* 14:163.
- Scheepmaker, J. W. A., Geels, F. P., Smits, P. H., and van Griensven, L. J. L. D. 1997. Control of the mushroom pests *Lycoriella auripila* (Diptera: Sciaridae) and *Megaselia halterata* (Diptera: Phoridae) by *Steinernema feltiae* (Nematoda: Steinernematidae) in field experiments. *Ann. Appl. Biol.* 131: 359-368.
- Schisler, L. C., Sinden, J. W., and Sigel, E. M. 1968. Etiology of mummy disease of cultivated mushrooms. *Phytopathology* 58:944-948.
- Schroth, M. N., Hildebrand, D. C., and Panopoulos, N. 2006. Phytopathogenic Pseudomonads and Related Plant-Associated Pseudomonads. Pages 714-740 in: *The Prokaryotes*. M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer, and E. Stackebrandt, eds. Springer, New York.
- Shirata, A., Sugaya, K., Takasugi, M., and Monde, K. 1995. Isolation and biological activity of toxins produced by a Japanese strain of *Pseudomonas tolaasii*, the pathogen of bacterial rot of cultivated Oyster mushroom. *Ann. Phytopathol. Soc. Jpn.* 61:493-502.
- Sinden, J. W. 1971. Ecological control of pathogens and weed molds in mushroom culture. *Annu. Rev. Phytopathol.* 9:411-432.
- Sinha, H., Pain, A., and Johnstone, K. 2000. Analysis of the role of *recA* in phenotypic switching of *Pseudomonas tolaasii*. *J. Bacteriol.* 182:6532-6535.
- Sivanesan, D. 2003. Diversity among bacteria causing blotch disease on the commercial mushroom, *Agaricus bisporus*. M.S. Thesis, Brock University, St. Catharines, ON, Canada.
- Siyoun, N. A., Surridge, K., van der Linde, E. J., and Korsten, L. 2016. Microbial succession in white button mushroom production systems from compost and casing to a marketable packed product. *Ann. Microbiol.* 66: 151-164.
- Soler-Rivas, C., Arpin, N., Olivier, J. M., and Wichers, H. J. 1999b. WLIP, a lipodepsipeptide of *Pseudomonas 'reactans'*, as inhibitor of the symptoms of the brown blotch disease of *Agaricus bisporus*. *J. Appl. Microbiol.* 86:635-641.
- Soler-Rivas, C., Jolivet, S., Arpin, N., Olivier, J. M., and Wichers, H. J. 1999a. Biochemical and physiological aspects of brown blotch disease of *Agaricus bisporus*. *FEMS Microbiol. Rev.* 23:591-614.
- Soloman, J. M., Beelman, R. B., and Bartley, C. E. 1991. Addition of calcium chloride and stabilized chlorine dioxide to irrigation water to improve quality and shelf-life of *Agaricus bisporus*. The Pennsylvania State University, University Park, PA.
- Sonnenberg, A. S., Baars, J. J. P., Gao, W., and Visser, R. J. F. 2017. Developments in breeding of *Agaricus bisporus* var. *bisporus*: progress made and technical and legal hurdles to take. *Appl. Microbiol. Biotechnol.* 101:1819-1829.
- Sparling, G. P., Farmor, T. R., and Wood, D. A. 1982. Measurement of the microbial biomass in composted wheat straw, and the possible contribution of the biomass to the nutrition of *Agaricus bisporus*. *Soil Biol. Biochem.* 14: 609-611.
- Stanier, R. Y., Palleroni, N. J., and Doudoroff, N. 1966. The aerobic pseudomonads: A taxonomic study. *J. Gen. Microbiol.* 43:159-271.
- Straatsma, G., Olijnsma, T. W., Gerrits, J. P., Amsing, J. G., op den Camp, H. J., and van Griensven, L. J. 1994b. Inoculation of *Scytalidium thermophilum* in

- button mushroom compost and its effect on yield. *Appl. Environ. Microbiol.* 60: 3049-3054.
- Straatsma, G., Samson, R. A., Olijnsma, T. W., Camp, H. J. M. O. D., Gerrits, J. P. G., and van Griensven, L. J. L. D. 1994a. Ecology of thermophilic fungi in mushroom compost, with emphasis on *Scytalidium thermophilum* and growth stimulation of *Agaricus bisporus* mycelium. *Appl. Environ. Microbiol.* 60:454-458.
- Suyama, K., Negishi, H., and Wakimoto, S. 2000. Selective medium for isolation of *Pseudomonas tolaasii*, the causal bacterium of bacterial blotch of cultivated mushrooms. *J. Agric. Sci.* 44:295-301.
- Svircev, A. M., Lehman, S. M., Sholberg, P., Roach, D., and Castle, A. J. 2011. Phage biopesticides and soil bacteria: multilayered and complex interactions. Pages 215-235 in: *Biocommunication in Soil Microorganisms*. Soil Biology. G. Witzany, ed. Vol. 23. Springer, Berlin, Heidelberg, Germany.
- Székely, A. J., Sipos, R., Berta, B., Vajna, B., Hajdú, C., and Márialigeti, K. 2009. DGGE and T-RFLP analysis of bacterial succession during mushroom compost production and sequence-aided T-RFLP profile of mature compost. *Microb. Ecol.* 57:522-533.
- Tolaas, A. G. 1915. A bacterial disease of cultivated mushrooms. *Phytopathology* 5:51-54.
- Tsukamoto, T., Murata, H., and Shirata, A. 2002. Identification of non-pseudomonad bacteria from fruit bodies of wild agaricales fungi that detoxify tolaasin produced by *Pseudomonas tolaasii*. *Biosci. Biotechnol. Biochem.* 66:2201-2208.
- Tsukamoto, T., Shirata, A., and Murata, H. 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* 39:273-278.
- Tsukamoto, T., Takeuchi, M., Shida, O., Murata, H., and Shirata, A. 2001. Proposal of *Mycetocola* gen. nov. in the family *Microbacteriaceae* and three new species, *Mycetocola saprophilus* sp. nov., *Mycetocola tolaasinivorans* sp. nov. and *Mycetocola lacteus* sp. nov., isolated from cultivated mushroom, *Pleurotus ostreatus*. *Int. J. Syst. Evol. Microbiol.* 51:937-944.
- Tsunedá, A., Suyama, K., Murakami, S., and Ohira, A. 1995. Occurrence of *Pseudomonas tolaasii* on fruiting bodies of *Lentinula edodes* formed *Quercus* logs. *Mycoscience* 36:283-288.
- Tu, C. C., and Liao, Y. M. 1989. Major diseases of cultivated mushroom and their control in Taiwan. *Mushroom Sci.* 12:615-626.
- Tyson, J., and Sockett, R. E. 2017. Predatory Bacteria: Moving from Curiosity Towards Curative Trends. *Microbiol.* 25:90-91.
- USDA NASS. 2018. Mushrooms. National Agricultural Statistics Service (NASS). Agricultural Statistics Board, United States Department of Agriculture (USDA), Washington, DC.
- Vantomme, R., Overstijns, A., Goor, M., Kersters, K., and De Ley, J. 1989. Routine diagnosis and sensitivity to chemical compounds of phytopathogenic and saprophytic pseudomonads from cultivated mushrooms. *Mushroom Sci.* 12:701-710.
- Vieira, F. R., and Pecchia, J. A. 2017. An exploration into the bacterial community under different pasteurization conditions during substrate preparation (composting – Phase II) for *Agaricus bisporus* cultivation. *Microb. Ecol.* 75:318-330.
- Vinatzer, B. A., and Bull, C. T. 2009. The Impact of Genomic Approaches on Our Understanding of Diversity and Taxonomy of Plant Pathogenic Bacteria. In: *Plant Pathogenic Bacteria: Genomics and Molecular Biology*. R. W. Jackson, ed. Caister Academic Press, UK.
- Vos, A. M., Heijboer, A., Boschker, H. T. S., Bonnet, B., Lugones, L. G., and Wösten, H. A. B. 2017. Microbial biomass in compost during colonization of *Agaricus bisporus*. *AMB Express* 7:12.
- Waltz, E. 2016. Gene-edited CRISPR mushroom escapes US regulation. *Nature* 532:293.
- Ware, W. M. 1933. A Disease of Cultivated Mushrooms Caused by *Verticillium Malthousei* sp. nov. *Ann. Bot.* 47:63-785.
- Weijn, A., Bastiaan-Net, S., Wichers, H. J., and Mes, J. J. 2013. Melanin biosynthesis pathway in *Agaricus bisporus* mushrooms. *Fungal Genet. Biol.* 55:42-53.
- Wells, J. M., Sapers, G. M., Fett, W. F., Butterfield, J. E., Jones, J. B., Bouzar, H., and Miller, F. C. 1996. Postharvest discoloration of the cultivated mushroom *Agaricus bisporus* caused by *Pseudomonas tolaasii*, *P. 'reactans'*, and *P. 'gingeri'*. *Phytopathology* 86:1098-1104.
- Wichmann, G., Sun, J., Dementhon, K., Glass, N. L., and Lindow, S. E. 2008. A novel gene, *phcA* from *Pseudomonas syringae* induces programmed cell death in the filamentous fungus *Neurospora crassa*. *Mol. Microbiol.* 68:672-689.
- Wiegant, W. M., Wery, J., Buitenhuis, E. T., and de Bont, J. A. M. 1992. Growth-promoting effect of thermophilic fungi on the mycelium of the edible mushroom *Agaricus bisporus*. *Appl. Environ. Microbiol.* 58:2654-2659.
- Wittebole, X., De Roock, S., and Opal, S. M. 2014. A historical overview of bacteriophage therapy as an alternative to antibiotics for the treatment of bacterial pathogens. *Virulence* 5:226-235.
- Wong, W. C., Fletcher, J. F., Unsworth, B. A., and Preece, T. F. 1982. A note on ginger blotch, a new bacterial disease of the cultivated mushroom, *Agaricus bisporus*. *J. Appl. Bacteriol.* 52:43-48.
- Wong, W. C., and Preece, T. F. 1979. Identification of *Pseudomonas tolaasii*: the white line in agar and mushroom tissue block rapid pitting tests. *J. Appl. Bacteriol.* 47:401-407.
- Wong, W. C., and Preece, T. F. 1980. *Pseudomonas tolaasii* in mushroom crops: a note on primary and secondary sources of the bacterium on a commercial farm in England. *J. Appl. Bacteriol.* 49:305-314.
- Wong, W. C., and 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. *J. Appl. Bacteriol.* 53:87-96.
- Wong, W. C., and Preece, T. F. 1985a. *Pseudomonas tolaasii* in cultivated mushroom (*Agaricus bisporus*) crops: effects of sodium hypochlorite on the bacterium and on blotch disease severity. *J. Appl. Bacteriol.* 58:259-267.
- Wong, W. C., and Preece, T. F. 1985b. *Pseudomonas tolaasii* in mushroom (*Agaricus bisporus*) crops. Bactericidal effects of six disinfectants and their toxicity to mushrooms. *J. Appl. Bacteriol.* 58:269-273.
- Wong, W. C., and Preece, T. F. 1985c. *Pseudomonas tolaasii* in mushroom (*Agaricus bisporus*) crops: activity of formulations of 2-bromo-2-nitropropane-1,3-diol (bronopol) against the bacterium and the use of this compound to control blotch disease. *J. Appl. Bacteriol.* 58:275-281.
- Wuest, P., and Bengtson, G. D. 1982. Penn State Handbook for commercial mushroom growers. The Pennsylvania State University, University Park, PA.
- Wuest, P. J. 1971. Use of dew point and ambient temperature differences to explain epidemic development of mushroom bacterial blotch. *Phytopathology* 61:918.
- Yabuuchi, E., Kosako, Y., Oyaizu, H., Yano, I., Hotta, H., Hashimoto, Y., Ezaki, T., and Arakawa, M. 1992. Proposal of *Burkholderia* gen. nov. and transfer of seven species of the genus *Pseudomonas* homology group II to the new genus, with the type species *Burkholderia cepacia* (Palleroni and Holmes 1981) comb. nov. *Microbiol. Immunol.* 36:1251-1275.
- Young, J. M. 1970. Drippy gill: a bacterial disease of cultivated mushrooms caused by *Pseudomonas agarici* n. sp. *N. Z. J. Agric. Res.* 13:977-990.
- Yun, Y. B., Kim, M. H., Han, J. H., and Kim, Y. K. 2017. Suppression of brown blotch disease by tolaasin inhibitory factors. *J. Appl. Biol. Chem.* 60:179-184.