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Destructive potential of some *Pseudomonas* species implicated as the causal agents of brown blotch disease in *Pleurotus ostreatus* (Jacq) P. Kumm

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ABSTRACT

The study evaluates the destructive potential of some *Pseudomonas* species implicated as the causal agents of brown blotch disease in *Pleurotus ostreatus* (Jacq) P. Kumm. The study was designed to isolate and identify the causal agents of the bacterial brown blotch of *P. ostreatus* and evaluate the effect of the pathogen on the mushroom. The *P. ostreatus* samples were inoculated with the infectious stock of the bacterial *Pseudomonas* species, the suspected causal agents of brown blotch in the mushroom. The results revealed that the symptoms and the eventual disease were confirmed by pathogenic study to be *Pserudomonas tolaasii*. The results revealed that the symptoms and the eventual disease were confirmed by pathogenic study to be *P. tolaasii*. The results also implicated *P. tolaasii* and *P. reactans* had a pathogenic interaction that led to the bacterial brown blotch in the mushroom; the condition which started as yellow stripe on the pilli was an indication of the presence of *P. reactans* in the infectious stock used to inoculate the healthy mushroom. And the later formation of reddish brown on the pilli down the stipe was also an indication of the presence of *P. tolaasii* that led to the collapse of the mushrooms. The above findings confirmed that the interactions of these species of *Pseudomonas* were actually the causal agents of the bacterial brown blotch disease in *Pleurotus ostreatus* (Jacq) P. Kumm.

Keywords: Pathogenic interactions, *Pserudomonas tolaasii, P. tolaasii, P. reactans, Pleurotus ostreatus* (Jacq) P. Kumm, brown blotch, infectious stock, mushroom pilli and Stipe.

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INTRODUCTION

Mushrooms are the fruiting bodies of higher fungi. They are macrofungi which are epigeous or hypogeous and are large enough to be seen with the naked eye unaided and can be picked by hand (Umechuruba and Elenwo, 1996; Fasidi et al., 2008; Nmom, 2021; Nmom et al., 2022). Mushrooms may mean different things to different people, having existed for millions of years with the imprints of their lamellae being found on woods and dating back before the origin of man. There are about 7000 species of mushrooms, but a little above 100 species are suitable for human consumption. A few species are commercially cultivated and the common edible strains belong to the *Agaricus, Lentinus, Flammulina, Volvariella, Tremella, Pholiota, Auricularia, Stropharia* and Tuber (Nmom, 2021).

Mushrooms are the richest source of vegetable proteins on a dry weight basis. Thus the percentage of proteins in mushrooms is much higher than that in cereals, pulses, fruits and vegetables. The protein in mushrooms contains all the essential amino acids and their quality is richer than that of eggs. They have good amounts of lysine amino acids, about 550 mg/g (Eger-Hummel, 1980; Fasidi and Olorunmaje, 1994). Edible mushrooms are usually picked up from the field or they are otherwise cultivated (Nmom, 2021).

Mushrooms, like any other crop, are often affected by a number of biotic and abiotic factors which may act singly or by interactive effects. Intensive cultivation of

mushrooms, such as *Pleurotus ostreatus* can be affected by such biotic and abiotic factors with microbial attacks inclusive (Bruno et al., 2013). *P. ostreatus* is a basidiomycete, often referred to as an oyster mushroom, distributed all over the world and since World War II, it has been commercially cultivated on a large scale (Piska et al., 2017). *P. ostreatus* was first cultivated in a subsistence measure in Germany during World War II and is now grown around the world. It belongs to the family Pleurotaceae (en.m.wikipedia.org). Intensive cultivations of *P. ostreatus* can be affected by some fungal molds, and viral and bacterial pathogens which cause dramatic production loss. The most ravaging microbe on the oyster mushroom is a bacterial infection (Nmom et al., 2022; Bahl, 1988; Jayakumar et al., 2010).

Bacteriosis in mushrooms is an unpredictable disease that occurs during the first and second sporophoral flushes causing great yield loss. Destructive levels are induced by environmental conditions, occurring at high relative humidity levels in growing chambers. The most common of them is the brown blotch caused by *Pseudomonas tolaasii* (Moquet et al., 1996). Brown blotch propagule, *P. tolaasii* colonizes the surface of the mushroom and spreads quickly due to the proximity of mushrooms on their beds. Favourable to the bacterial blotch outbreak is excessive water in the casing layer and low aeration rate in the growing house. These conditions can induce the occurrence of morphological variants or aggressive pathovars of *P. tolaasii; P. reactans* in interactions and synergy (Demangee et al., 1990).

Brown blotch disease of *P. ostreatus* is a bacterial infection with a wide range of pathogenicity on *Agaricus, Flammulina,* Shiitake and oyster mushrooms. The pathogen is able to survive in a variety of environmental conditions and competes favourably with other bacterial populations because it has several biological mechanisms to survive (Tajalipour et al., 2014). The infecting bacterium, *P. tolaasii* colonizes the surface of the host mushroom and produces a toxin, known as tolaasin with which it disrupts the plasma membrane of the mushrooms causing them to collapse. When the tolaasin toxin is secreted, it infiltrates the deeper hyphal tissues of the mushroom (Nmom et al., 2022; Soler-Rivas et al., 1999). Hutchinson and Johnstone (1993) reported that tolaasin is an effective biosurfactant that decreases water tension. They also added that low water tension causes bacterial spores to spread across large areas (Piska et al., 2017). It has also been reported that *P. tolaasin* uses a biological mechanism to carry out its pathogenesis; such as its ability to switch between a smooth and rough phenotypic strain in optimal conditions. The pathogen undergoes exponential growth and increases the production of the toxin (Solar–Rivas et al., 1999). It has also been reported that *P. tolaasin* has a synergistic interaction with *P. reactans* in the etiology of brown blotch disease of oyster mushrooms.

Based on the foregoing, this study aimed to isolate and identify the causal agents of the bacterial brown blotch disease of *P. ostreatus* and evaluate the effect of the disease on the mushroom.

MATERIALS AND METHODS

Preparation and culture of infectious stock

The study utilized serial dilutions which were carried out in four parts by spread plating. An aliquot, (0.1 ml) of the sample (of the Mushroom tissue infected with brown blotch disease) was used to inoculate the media which was later incubated and counted. This was severally subcultured to obtain pure cultures and a series of biochemical tests were carried out.

In preparing the media with nutrient agar, 28 g of the nutrient agar was weighed into a 1000 ml Erlenmeyer flask; which was brought to a boil to dissolve completely by heating over a Bunsen burner flame for 30 min. The medium was sterilized at 121°C for 15 min at 15 psi.

This was allowed to cool to 45°C and 15 ml of the medium was poured into sterile Petri dishes which were allowed to set and dry in an oven before use.

The preparation of normal saline was done by weighing 8.5 g of sodium chloride and dissolved in 1000 ml of distilled water. Nine milliliters (9 ml) of the solution was dispensed into various test tubes, cooked and then sterilised by autoclaving at 121°C for 15 min and at 15 psi.

Tenfold serial dilution was carried out for the isolation of bacteria from the sample and 1 g of the sample was weighed 9ml prepared normal saline as 10^{-1} dilution. 1 ml was transferred from the initial test tube into 9ml sterile normal saline in a second test tube as 10^{-2} dilution, etc as described by Holt et al. (1994), Cheesbrough (2006) and Nrior et al. (2021).

Enumeration and Isolation of infectious microorganisms

In the enumeration and isolation of microorganisms; an aliquot (0.1 ml) of the sample was transferred into sterile agar plates in duplicates, uniformly spread with sterile glass spreader (or Spread plate method) and incubated in an inverted position at 37°C for 24 h for bacteria and room temperature for a period of 2 to 7 days for fungi. After incubation, the plates were observed and the colonies that developed were counted and recorded.

Identification of the isolates was based on their colonial morphology and microscopic examination as well as the biochemical tests. Morphological studies were carried out on different media plates used for the isolation of the microbes; pure colonies were isolated based on colony size, shape, pigmentation, elevation and texture of the individual organisms after 48 h of growth at 30°C. Pure isolates from the respective media were characterised and identified based on their morphological, biochemical

and physiological features according to Holt et al. (1994), Cheesbrough (2006) and Nrior et al. (2021).

In colonial morphology, a colony of the isolate was picked and streaked on the freshly prepared nutrient agar and incubated at 37°C for 24 h. After incubation, morphological features; such as shape, colour, size, edge, texture and elevation of the colony of the isolate were observed visually with a hand lens. The overnight pure culture was used to determine the cell morphology: motility test and gram reactions.

Experimental set-up

A set-up containing 6 apparently healthy *Pleurotus ostreatus* mushrooms which were established at Dilomat farms and services, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, and were infected with the 6 isolates from the test above (Figure 1). They were monitored closely for significant changes and possible repetition of the synergy between *P. tolaasii* and *P. reactans* as earlier reported (Demangee et al., 1990).

Figure 1. Apparently healthy mushrooms before inoculation with an infectious stock of bacterial brown blotch disease.

RESULTS AND DISCUSSION

The results of this study revealed that one day after inoculation of the apparently healthy growing mushroom on their beds, the edges and centre of the pilli developed yellow colourations and 3 days later, the mushroom pilli began to turn reddish brown and as the disease progressed by the day, the infection also progressed into the stipe and took over the entire mushroom. As the infection continued, the mushrooms lost their integrity and consistency on the substrate. These developments led to the collapse of the mushroom. These are shown in Figures 2 to 4.

The results of this study revealed that bacteriosis in mushrooms is unpredictable and occurs during the first and second flushes and sometimes after harvest. The environmental conditions of the place of this experiment had a relatively high humidity which may have predisposed the agents of the disease to spread fast. This seems to be in line with the suggestions of Moquet et al. (1996); who suggested that high relative humidity and sufficient water continuously on the casing layer favours the outbreak of the bacterial brown blotch disease in *P. ostreatus.* The speed and progress of the infection and symptoms resembled the reported infection caused by *P. tolaasii,* the causal agent of the disease. The symptoms recorded in this study agree with the report from Nmom et al. (2022).

It seems that the condition of excessive water in the casing layer induced the quick infection of the inoculated mushrooms which also seems to agree with the report of Demangee et al. (1990) who suggested that favourable to the bacterial brown blotch disease is excessive water in the casing layer and low aeration rate in the mushroom cropping house; the conditions which they suggested induces the occurrence of the morphological variant or aggressive pathovars of *P. tolaasii* and *P. reactans* in interactive synergy. The way the inoculated sample got

Figure 2. Initiation of infection, a day after inoculation with infectious stock.

Figure 3. Infection shown on the tissues of the mushroom pileus.

quickly infected in a short while sends a signal to the report of Lo Cantore and Lacobellis (2014); who submitted that the etiology of lesions on cultivated *P. ostreatus* involves a complex, composed of the interactions between *Pseudomonas tolaasii* and *P. reactans*. The results of this study truly align with their findings. This is because the first symptom observed was yellowish stripes or (lesions) on the edges of the mushroom pilli. It then suggests that the first agent to attack the mushroom was *P. rectans* in line with the scholar's report that *P. tolaasi* is consistently associated with reddish-brown blotches on *P. ostreatus* tissues; while *P. reactans* is mostly associated with superticial yellow lesions on the sporocarps (i.e. the Pilli); which in pathogenicity assays have altogether caused discolouration and subsequent brown blotching of the whole mushroom tissue and their associated collapse.

The quick collapse of the mushroom seems to be that they lost the supply of nutrients from the substrates. This also agrees with the report of Tajalipour et al. (2014) that *Pseudomonas* species are able to compete for the available nutrients with the growing mushrooms; hence, it has several biological mechanisms to survive.

It had earlier been reported that brown blotch pathogen spreads quickly, causing huge damage as confirmed with the findings of this study, confirming the suggestions by Nmom et al. (2022) to be real; because the reports suggested that the pathogen produces a toxin known as

Figure 4. The whole body of the mushroom became infected and the mushroom losing integrity on the substrates collapsed.

tolaasin which causes brown spots covering the surface of the mushroom as was recorded in this study. It could also be that the pathogen employed a mechanism of infection by synergy as Hutchinson and Johnstone (1993) reported that the infecting *P. tolaasii* colonizes the mushroom surface and releases the toxin tolaasin which disrupts the plasma membrane of the mushroom; causing the tissues to collapse. Little wonder, why the mushrooms lost their integrity, consistency and turgidity and consequently became flaccid, ready to tear; all because of the effect of the toxin which as they reported, infiltrated the deeper mushroom hyphal tissues. This supports the suggestions of Solar-Rivas et al. (1999), that tolaasin is an effective biosurfactant that reduces water tension and that low water surface tension causes the bacterial spore to spread across large areas.

The rate at which the inoculated mushroom got infected seems to agree again with the suggestions of Solar–Rivas et al. (1999) who disclosed that *P. tolaasii* has a biological mechanism to carry out its pathogenesis through its ability to switch between a smooth and rough phenotypic strain and has the optimal conditions in which its population could undergo exponential growth; increasing the production of the toxin tolaasin. This is the true state of the process of the infection by inoculation in this study which is also in line with the emphasis of the report obtained from the Extension PSU.edu. The two bacterial strains worked in interactive synergy to bring about the bacterial brown blotch disease in the oyster mushroom, *Pleurotus ostreatus* in the present study.

Conclusions

This study established that Brown Blotch of *Pleurotus ostreatus* is caused by a bacterial genus, *Pseudomonas*, made up of many strains and the particular strain that causes Brown Blotch is *P. tolaasii.* From the study, it is asserted that the low humidity environment of the mushroom house is favourable for the fast spreading of the pathogen across the mushroom in the farm by the bacterium genus.

An interesting aspect of the infection of *P. tolaasii* on *P. ostreatus* is that there is an interactive synergy between the two strains; *P. tolaasii* and *P. reactans* to bring about successful infection of the mushroom and render the fruit bodies toxic as tolaasin turns the fruiting bodies leathery and tearing thorny on touch.

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