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# BACTERIAL BLOTCH OF OYSTER MUSHROOM CULTURES IN PUERTO RICO

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**ABSTRACT.** Moist lesions were observed on oysters mushroom basidiocarps. The lesions were yellow, watersoaked and soft. Proliferating lesions spread all over the basidiocarp causing premature deterioration and death. Basidiocarp with symptoms were cut and surface desinfested by diluted bleach (10%). Pieces were then plated in tryptic soy agar (TSA). Symptomatic pieces of basidiocarps were placed in 2 ml of sterile distilled water, teased and streaked on TSA medium plates. Plates were incubated for 48 hours at 28EC. Single colonies forming units, were isolated and purified in TSA and King's B medium.

Koch's postulates were completed by inoculating the healthy oyster mushrooms with purified cultures. Bacterial culture broth containing approximately $10^9$ cfu $ml^{-1}$, was placed on the basidiocarp surface. Controls were made with sterile distilled water. They were placed in lidded glass dishes making a humidity chamber, and incubated at 28°C. Treatments were made in duplicates. Characteristic lesions and symptoms, were observed 24 hrs. after inoculation. Any symptom was observed in controls. The test bacteria was then re-isolated as previously described.

The API Rapid NFT and BIOLOG, an automatic identification system were performed for the identification of the isolated bacteria. The isolated bacteria was identified as *Burkholderia cepacia.*

## INTRODUCTION

Oyster mushroom (*Pleurotus* spp.) are edible mushrooms that have been cultivated commercially. Worldwide oyster mushroom increased production from 169,000 metric tons in 1987 to 909,000 in 1990 (Stamets, 1993). The University of Puerto Rico, Mayaghez Campus, performed experiments with species with tropical adaptation and potential. Several diseases has been reported in *Volvariella volvacea* and *Pleurotus ostreatus* cultures, caused by other fungi and insects (Almodovar, 1989; Barreto-Bosques, 1992; Dones, 1990; Rivera-Vargas, 1985). Edible mushroom diseases have been reported all over the world where mushroom are cultivated. Bacteria is one of the pathogens that attack and deteriorate edible mushroom, seriously. *Pseudomonas* spp. are the most common bacteria reported in edible mushrooms (Fahy and Persley, 1983).

*Pseudomonas agarici* produces drippy gill of mushroom (*Agaricus bisporus*), causing formation of droplets of pure colonies of mucoid, glistening bacteria, which develop in the surface of the gills within a dark brown to black, watersoak spot about 2mm in diameter. *Pseudomonas tolaasii* produces bacterial blotch, a serious disease of cultivated mushroom (*A. bisporus and A. bitorquis*). Symptoms are light to dark brown, sunken lesions appearing on caps during all stages of growth or in storage. *Pseudomonas* sp. caused mummy disease of cultivated mushroom frequently fail to develop perfect caps and may be distorted (Fahy and Persley, 1983).

In Puerto Rico, Hepperly and Ramos-Dávila (1986) reported *Volvariella volvacea*, straw mushroom, having a basal rot caused by *Pseudomonas aeruginosa.* They reported that the

pin to egg stages show mycelium softening, discoloration shrinkage and watersoaking. Basidiocarp in button and egg stages become distinctly beaked.

In our laboratory, moist lesions were observed on oysters mushroom basidiocarps. The lesions were yellow, watersoaked and soft. Drops of exudate were observed with bacterial swarming. Proliferating lesions spread all over the basidiocarp and caused premature deterioration and death of the fungi. Yield and quality losses due to this condition varied between 10 and 20%. It was most prevalent on production units that were exposed to higher humidity (RH>95%) during primordia and basidiocarp development. Temperature in the production rooms was 26±2° C. Mushrooms stored in the refrigerator, also showed blotch symptoms.

## MATERIALS AND METHODS

Basidiocarp with symptoms were cut and surface desinfested by diluted bleach (10%). Pieces were then plated in tryptic soy agar (TSA). Other pieces of symptomatic basidiocarps were placed in 2 ml of sterile distilled water, teased and streaked with a sterile loop on to TSA medium plates. Plates were incubated for 48 hours at 28°C. Single colonies forming units, were isolated and purified in TSA and King's B medium.

Isolated bacteria was assayed for pathogenicity test. Koch's postulates were completed by inoculating the healthy oyster mushrooms with purified aseptic cultures. A drop of 25 µl of the bacterial culture broth containing approximately $10^9$ cfu $ml^{-1}$, was placed on the basidiocarp surface. Controls were made with sterile distilled water. Inoculated mushrooms were placed in lidded glass dishes making a humidity chamber, and incubated at 28°C for three days. All treatments were made in duplicates.

The API Rapid NFT and BIOLOG, an automatic identification system, were performed for the identification of the isolated bacteria.

## RESULTS AND DISCUSSION

Bacterial growth was isolated from lesions on oyster mushroom basidiocarp. Only one type of colony with the same visual morphological characteristic, was observed. Colonies on TSA were smooth, cream-yellow, and slightly translucent. Colonies on King's B were yellow and opaque, wrinkled and slightly elevated. King's B medium turned light yellow. No fluorescence was produced. Gram stain revealed gram negative rods.

Pathogenicity test showed characteristic lesions and symptoms, on basidiocarps that were observed 24 hrs. after inoculation. Lesions were yellow, soft and watersoaked and spreaded all over the basidiocarps. Any symptom was observed in controls. The test bacteria was then re-isolated as previously described.

Similar blotch disease during mushroom production caused by pseudomonads species, have been reported (Cutri et al., 1984; Goor et al., 1986; Wells et al., 1996). Bacterial blotch disease reduce crop yield, because lesions develop and deteriorate basidiocarps, rapidly. It is considered the most important bacterial disease of mushroom.

The bacteria was identified by using the API Rapid NFT and BIOLOG methods. From these tests, the isolated bacteria was identified as *Burkholderia cepacia (=Pseudomonas cepacia)*. *B. cepacia* appeared to be pathogenic to mushroom causing blotch symptoms.

## CONCLUSIONS

Bacterial blotch affected the mushrooms basidiocarps and decrease the crop yield. *Burkholderia cepacia* caused bacterial blotch of oyster mushroom in culture.

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