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SHORT NOTES

First report of brown discoloration of *Agaricus bisporus* caused by *Pseudomonas agarici* in southern Italy

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Summary. From superficial brown discolorations on the caps and stalks of deformed sporophores of *Agaricus bisporus*, bacteria were consistently isolated. On the basis of biochemical, nutritional and pathogenic characteristics, both on tissue blocks and on whole sporophores of *A. bisporus,* the above bacteria were identified as strains of *Pseudomonas agarici*.

Key words: brown blotch, *Pseudomonas tolaasii*, *Pseudomonas reactans*, cultivated mushrooms, bacterial diseases.

Introduction

Several bacteria, in particular fluorescent pseudomonads, attack different species of cultivated mushrooms causing high crop losses (Fermor, 1986; Gill, 1995). Among these one of the most dangerous on cultivated *Agaricus* spp. is *Pseudomonas tolaasii,* the causal agent of brown blotch (Tolaas, 1915; Paine, 1919). This disease causes dark brown, often wet and sunken lesions on the caps and stalks, which render the crop unmarketable. Two other bacterial species that are associated with species of cultivated mushrooms, in particular *Agaricus bisporus* (Lange) Imbach, are *P. gingeri*, the still not classified causal agent of ginger blotch (Wong *et al*., 1982), and *P. agarici*, which causes a disease called drippy gill (Young, 1970). Ginger blotch is characterised by small, pale yellowish-brown blotches on the sporophores, which turn reddish-ginger as the mushrooms mature. In the case of drippy gill, the pathogen attacks the gills, after the inner veil breaks down, and the mushrooms do not develop, or their development is delayed and they become distorted. More recently (Geels *et al*., 1994), *P. agarici* was reported in The Netherlands as being also the causal agent of a highly damaging disease of *A. bisporus* called brown discoloration, which is characterised by a superficial brown discoloration of the sporophores. Drippy gill, unlike brown discoloration (Geels *et al*., 1994), is a rare disease, though in some mushroom houses it may be intense (Gill, 1995). In addition, *P. agarici* also causes yellow blotch of *Pleurotus ostreatus*, observed for the first time in California in 1983 (Bessette *et al*., 1985).

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Fig. 1. Superficial brown discolorations on both caps and stalks of *Agaricus bisporus* sporophores.

Superficial brown discolorations on the caps and stalks of deformed *A. bisporus* sporophores (Fig. 1) were recently noticed in a mushroom house in Apulia. Since the attack was severe, and looked as if it was due to a bacterial infection, it was decided to investigate the causal agent of the alterations. Here we report for the first time in Italy, the identification of *P. agarici* as the causal agent of brown discoloration of *A. bisporus*.

Materials and methods

Bacterial isolation

Bacteria were isolated from sporophores of *A. bisporus* following the method of Lelliott and Stead (1987). Pure cultures were maintained on slopes of nutrient agar plus 2% glycerol (NAG) at 4°C, whereas for inoculum preparation bacterial isolates were grown on medium B of King (KB, King *et al*., 1954) at 25°C for 48 h. For long-term storage, isolates USB78, USB84 and USB88, further characterised for pathogenicity, the white line test, and nutritional and biochemical features, were lyophilised.

Bacterial strain characterisation

Bacterial isolates were assayed for pathogenicity on tissue blocks and on fresh-picked whole sporophores of *A. bisporus* following methods previously described (Ercolani, 1970). Control sporophores were inoculated with sterile distilled water (SDW) or with the type strains NCPPB2289 of *P. agarici* and NCPPB2192 of *P. tolaasii*.

The white line test was performed following the procedure of Wong and Preece (1979) with minor modifications. Strains NCPPB1311 of *P. reactans* and NCPPB2192 of *P. tolaasi,* were used as controls.

Bacterial isolates were evaluated for LOPAT characteristics (Lelliott and Stead, 1987) and for the differential nutritional characteristics of bacterial pathogens associated with cultivated mushrooms (Goor *et al*., 1986). Strains NCPPB2289, NCPPB2472 (*P. agarici*), NCPPB2192 (*P. tolaasii*), NCPPB1311 (*P. reactans*), and NCPPB2874 (*Pseudomonas* spp.) isolated from mushrooms showing Mummy disease symptoms were used for comparison (Tucker and Routien, 1942; Fletcher *et al*., 1989).

The nutritional profile of strain USB78 was evaluated with the computer-assisted system Biolog (Biolog, Inc., Hayward, CA, USA).

Results and discussion

Bacterial isolations from superficial brown spots and streaks on *A. bisporus* sporophores were always positive and these bacteria gave rise to colonies which mostly showed the typical morphology of *P. agarici* on nutritive agar after 5 days of incubation at 25°C: circular, two mm in diameter, domed, whitish, with a buttery consistency.

All the isolates, as well as the type strain NCPPB2289 of *P. agarici,* caused browning of the tissue blocks of *A. bisporus*, although the degree of browning was less than that caused by the strains of *P. tolaasii* used as control (Fig. 2). Unlike the control strains, isolates of *P. agarici* never caused sunken lesions and rotting of the mushroom tissues. The pathogenicity test of *P. agarici* strains on whole sporophores was positive and here too, only superficial brown discoloration of the inoculated caps were caused, unlike what occurred with the *P. tolaasi* strains (Fig. 3). Under the same test conditions, strain NCPPB2192 of *P. tolaasii* caused sunken brown lesions.

Bacterial isolates that were positive in the pathogenicity tests were Gram negative, produced flu-

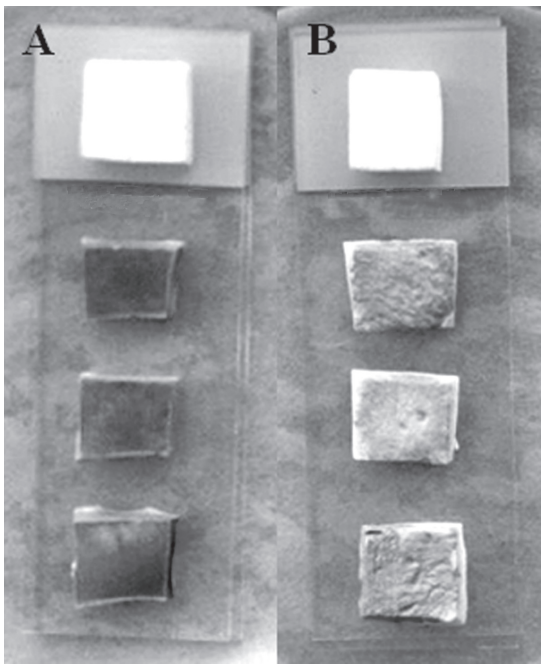

Fig. 2. Deterioration of *Agaricus bisporus* tissue blocks inoculated with 10 ml drops of *Pseudomonas tolaasii* NCPPB2192 (A) or with *P. agarici* USB78 (B) 10^8 cfu ml^{-1} suspensions. The tissue blocks in upper rows were treated with sterile distilled water.

orescent pigments when grown on KB, and did not form white precipitates in the white line test when grown near strains NCPPB2192 of *P. tolaasii* or NCPPB1311 of *P. reactans*.

Bacterial isolates showed the LOPAT characteristics of fluorescent pseudomonads of group III (Lelliott and Stead, 1987), to which *P. agarici* belongs.

Bacterial isolates did not grow on sorbitol, erythritol, L-arabinose, L-rhamnose, L-arabitol, 2-ketogluconate, *n*-valerate, D-tartrate or histamine. The above nutritional pattern, which is typical of *P. agarici* strains (Goor *et al*., 1986), was also shown by strains NCPPB2472 and NCPPB2289 of *P. agarici* used as controls. The identity of some of the above strains was confirmed by the nutritional profile obtained with the Biolog computer-assisted system. Strain USB78, identified as *P. agarici*, was deposited at the International Collection of Micro-organisms from Plants, Auckland, New Zealand, and designated as ICMP13775.

In conclusion, the results confirm that *P. agarici* was the causal agent of the brown discoloration

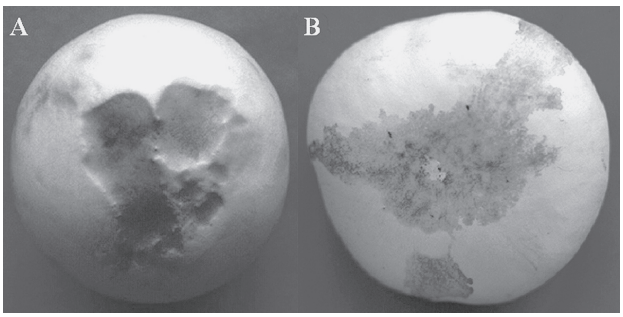

Fig. 3. Symptoms on whole sporophores of *Agaricus bisporus* inoculated with 10 ml drops of *Pseudomonas agarici* USB78 (A) or with *P. tolaasii* NCPPB2192 (B) 10^8 cfu ml^{-1} suspensions.

observed on *A. bisporus* sporophores. To the authors knowledge this is the first report of this disease in Italy.

As in the case of the disease observed by Geels *et al*. (1994) in The Netherlands, the typical symptoms of drippy gill caused by *P. agarici* (Young, 1970) were not observed. This suggests that the pathogen produces different symptoms in different environments.

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