



Symptomatology and Microecology of *Pseudomonas tolaasii* Infection in Button Mushroom

Mahsa Moallem¹, Ebrahim Osdaghi^{1*}

¹Department of Plant Protection, College of Agriculture, University of Tehran, Karaj, Iran.

Abstract

Brown blotch disease caused by *Pseudomonas tolaasii* severely affects cultivated mushrooms. Pathogenicity tests on *Agaricus bisporus* caps showed typical brown blotch symptoms within 48 h, while controls remained healthy. FE-SEM revealed dense bacterial attachment to hyphae, along with membrane degradation and pore-like damage. These results demonstrate that *P. tolaasii* causes disease through hyphal colonization and membrane disruption, leading to tissue dysfunction.

Introduction

Mushroom physiology involves tightly regulated cellular processes, including membrane integrity and stress responses, which are highly sensitive to microbial interactions. Among bacterial diseases of cultivated mushrooms, brown blotch caused by *Pseudomonas tolaasii* is one of the most destructive, producing characteristic brown lesions on *Agaricus bisporus* caps and leading to major economic losses. Disease development is driven not by mechanical invasion but by physiological disruption of host cells, primarily through the action of tolaasin, a pore-forming lipodepsipeptide toxin that damages plasma membranes and compromises hyphal integrity. Successful pathogenicity depends on bacterial attachment to mushroom hyphae, enabling localized toxin activity. In this study, pathogenicity assays and field-emission scanning electron microscopy (FE-SEM) were used to evaluate *P. tolaasii* infection and to link bacterial colonization with ultrastructural and physiological damage in mushroom tissues.

Materials and methods

The type strain *Pseudomonas tolaasii* CFBP 2068^T, originally isolated from *Agaricus bisporus*, was obtained from the CIRM-CFBP culture collection and grown on standard bacteriological media. Bacterial suspensions were prepared following established protocols and stored short- and long-term under appropriate conditions.

Pathogenicity was assessed on fresh, healthy mushroom caps using the cap-cut method. Mushroom caps were inoculated with a standardized bacterial suspension and incubated under humid, dark conditions. Symptom development was monitored for 48 h, and Koch's postulates were confirmed by re-isolation and identification of the bacterium. Experiments were repeated three times.

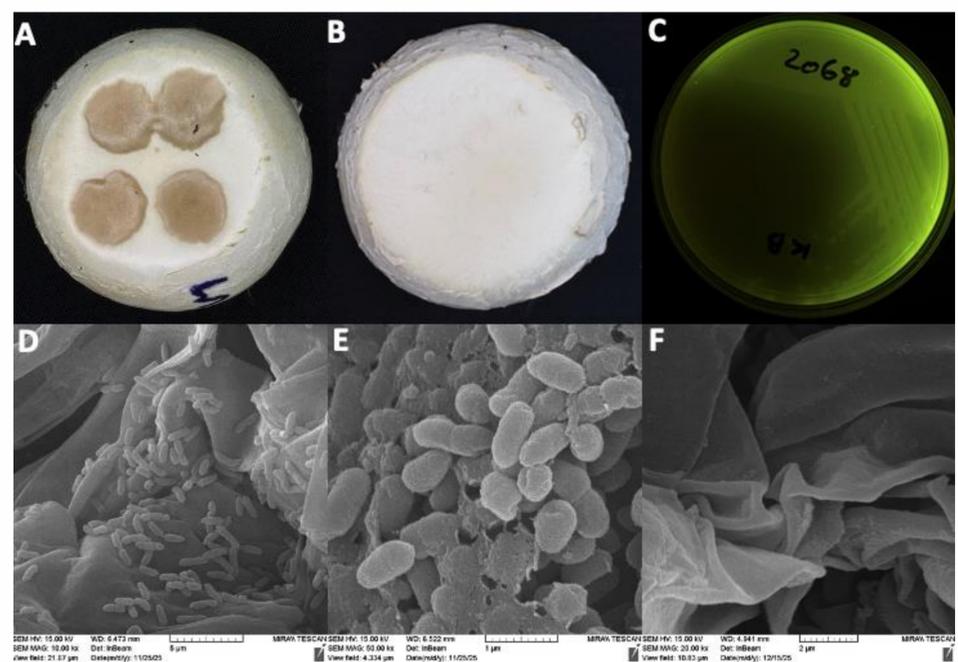
Ultrastructural changes in infected mushroom tissues were examined using field-emission scanning electron microscopy (FE-SEM). Symptomatic cap tissues were chemically fixed, dehydrated, gold-coated, and observed at high magnifications to evaluate bacterial colonization and host tissue damage.

Results and discussion

Pseudomonas tolaasii caused typical brown blotch symptoms on *Agaricus bisporus* caps, beginning as yellowish-brown discoloration and intensifying within 48 h. No symptoms were observed in negative control caps treated with sterile water (Fig. 1A–C).

Scanning electron microscopy revealed extensive bacterial colonization on infected hyphae, with dense attachment of *P. tolaasii* cells to the hyphal surface (Fig. 1D–E). Infected tissues showed clear ultrastructural damage, including membrane degradation and pore-like lesions, which were absent in control samples (Fig. 1F).

These observations support that disease development is not due to mechanical tissue invasion but results from membrane disruption caused by the pore-forming toxin tolaasin. Bacterial attachment to hyphae appears essential for localized toxin activity and subsequent physiological collapse of mushroom tissue. Overall, SEM evidence directly links bacterial colonization with structural and physiological damage underlying brown blotch disease.



References

- 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*. *Physiological and Molecular Plant Pathology*, 39(1), 57-70. [https://doi.org/10.1016/0885-5765\(91\)90031-C](https://doi.org/10.1016/0885-5765(91)90031-C)
- Schaad, N. W., Jones, J. B., and Chun, W. (eds.). (2001). *Diagnostic Procedures for Bacterial Plant Pathogens*, 3rd Edition. St. Paul, MN: APS Press.
- Tolaas, A. G. 1915. A bacterial disease of cultivated mushrooms. *Phytopathology* 5:51-54.