Unraveling the mycosphere microbiome: bacterial diversity in *Pleurotus cystidiosus* substrates

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Mushrooms are in high demand in Sri Lanka; however, their market availability remains limited due to specific environmental conditions, particularly within the mushroom substrate. Mushroom substrates host a complex microbiome, with over 90% of the microbial biomass consisting of bacterial and fungal species. Understanding the beneficial and antagonistic dynamics within these microbial communities presents innovative opportunities to enhance bacterial–fungal interactions (BFIs) to promote mushroom growth. The present study used culture-dependent methods to isolate, identify, and characterise bacterial species from *Pleurotus cystidiosus*' mycosphere, assessing their potential to enhance host growth. A range of selective and general media, including King's B, tryptic soy agar, and Reasoner's two agar, was used to isolate bacteria. Additionally, the physicochemical parameters of the substrates were analysed to assess quality and composition. Morphological characterisation yielded a total of eleven bacterial isolates across the media, and five bacterial species were identified through morphological characteristics observed under stereo and upright microscopy, Gram staining, and colony imaging. Molecular identification via amplification of 16S rRNA revealed the presence of *Bacillus tequilensis*, and *Niallia nealsonii* as well as facultative anaerobes *Franconibacter helveticus* and *Staphylococcus pasteuri*. This study provides evidence of growth-promoting bacterial communities within the native mycosphere of *P. cystidiosus* and their potential role in enhancing mushroom cultivation by increasing biomass without disrupting the native microbiome. Future research will focus on analysing the functional roles of these bacteria and developing bioinoculants to optimise beneficial BFIs for sustainable mushroom farming.

Keywords: *Bacillus tequilensis*, bacterial–fungal interactions, mycosphere microbiomes, *Pleurotus cystidiosus*.

Sri Lanka, among many other cultures, has a history of using wild mushrooms for food and medicine. Despite the rich history, only a limited number of studies have systematically explored their edibility, medicinal applications, and health benefits[1–3]. Commercial mushroom cultivation in Sri Lanka began in 1985 with the United Nations Development Programme support[4], yet the industry still faces challenges in achieving large-scale growth and sustainability. The most demanded edible mushroom genera include *Agaricus*, *Pleurotus*, *Lentinula*, *Volvariella*, and *Auricularia* worldwide[5]. Within the genus *Pleurotus*, the abalone mushroom (*Pleurotus cystidiosus*) is rich in protein, fibre, vitamins, and minerals, making it a functional food[6]. It contains bioactive compounds, e.g. alkaloids, phenols, flavonoids, β-carotene, and lycopene[7] with antioxidant, immunomodulatory, and anti-carcinogenic activities[8]. Mushroom cultivation is a biotechnological process that requires controlled environmental conditions for optimal growth. This process includes several key stages, namely strain selection, substrate preparation, inoculation, incubation, fruiting, and harvesting[2]. Cultivation techniques vary by species and include solid-state fermentation, submerged fermentation, and bag cultivation[9]. While traditional log cultivation is still practiced for wood-decomposing fungi, modern methods emphasise sterile techniques and controlled environmental parameters (humidity, temperature, and CO_2 levels) to maximise yield and quality[10,11]. The choice of spawning technique, whether single-layer spawning, double-layer spawning, or mixed spawning, significantly influences mycelial colonisation and subsequent mushroom production[12].

The complexity of the growing substrate fosters a dynamic microbial ecosystem that includes bacteria that can facilitate growth, known as mushroom growth-promoting bacteria (MGPB)[13]. Previous studies identified *Pseudomonas putida* to be capable of promoting mushroom production[14], and *Pseudomonas fluorescens* and *Bacillus megaterium* are capable of enhancing mycelial development and substrate decomposition, improving mushroom production[15]. Furthermore, nitrogen-fixing bacteria *Azospirillum lipoferum* have been shown to significantly increase mushroom yield, while phosphorus and potassium-solubilising bacteria, such as *B. megaterium* and *Frateuria aurantia*[16], contribute to improved cap firmness and extended post-harvest shelf life.

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