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Anti- quorum sensing activity of an oyster mushroom, *Pleurotus florida* (Mont). against *Pseudomonas aeruginosa*

P. Silambarasan, R.Sumathy* and R.Kumuthakalavalli

Department of Biology, The Gandhigram Rural Institute, Deemed University, Gandhigram, Dindigul.

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ABSTRACT

Pseudomonas aeruoginosa, a bioreporter strain was used as an indicator to monitor quorum-sensing inhibition. Quorum sensing (QS) is a system of stimulus and response correlated to bacterial population density. The increasing incidence of multi-drug resistant pathogens has stimulated the search for novel anti-virulence compounds. Although many phytochemicals show promising anti microbial activity, their power lies in their anti-virulence properties. It is hypothesized that many naturally occurring terrestrial plants, algae and fungi traditionally used as medicines may also produce anti-QS compounds. To test this hypothesis, *Pleurotus florida*, the oyster mushroom was assessed qualitatively and quantitatively for its anti-QS activity using *Pseudomonas aeuroginosa*. A special swarm media that allow swarming motility growth of *Pseudomonas aeuroginosa* was used to conduct inhibition of swarm motility assay using mushroom extract. The methanol and chloroform extracts of the mushroom was tested for their inhibition of AHL (Acyl-Homoserine Lactone) production and biofilm formation. Inhibition of AHL for methanol extract ranged from 37.89-58.94% and for chloroform extract ranged from 50.05-70.05% at a concentration of 100-500µg/ml. Inhibition of biofilm formation for methanol extract ranged from 33.9-83.9% and for chloroform extract ranged from 60.07-82.1% at a concentration of 100-500µg/ml. The results of this study provided evidence that organic solvent extracts of the mushroom *Pleurotus florida* exhibited appreciable antiquorum sensing property and it justifies their use in the traditional medicine for the treatment of different multi-drug resistant microbes.

Introduction

Virulent factors produced by pathogens play an important role in the infectious process, which is regulated by a cell-to-cell communication mechanism called quorum sensing (QS). Communications among bacteria is achieved via, the production, diffusion, detection and responses to chemical signaling molecules known as autoinducers. When a threshold concentration is reached the autoinducers are detected and this leads to quorum sensing, by regulation of bacterial behaviors such as formation and release of virulence factors, antibiotic production and also biofilm formation (Redfield, 2002). Bacterial intercellular communication or quorum sensing controls the pathogenesis of many medicinally important microorganisms. The incidence of multi drug resistant pathogenic bacteria is increasing worldwide, and this has rendered current antibiotic regimes ineffective in many cases. Due to the extensive emergence of antibiotic resistant bacteria there is a rising need to control this drug resistance. Many opportunistic pathogenic bacteria depend on QS system to coordinate their virulence expression that may lead to the production of QS signal molecules and eventually the development of chronic bacterial infections (Chong Lek Koh et al., 2013). Thus, interference with QS has been regarded as the novel way to control bacterial infection.

Pseudomonas aeuroginosa, a bioreporter strain is an intrinsic gram negative bacterium which is a well studied model for anti-QS. It is an important opportunistic human pathogen, which causes infections in patients with compromised immune systems and cystic fibrosis. The pathogen uses quorum sensing to coordinate behaviors such as biofilm formation, swarming motility and aggregation through formation of signal molecule i.e., AHL. The QS can be targeted in a number of ways, including inhibition of AHLs, biofilm formation and swarming motility, evidence is provided that swarming motility of the bacterium is quorum sensing regulated which is formed by surface translocation (Avantika Lal, 2009).

Mushrooms were employed initially because of their therapeutic values in traditional medical practices, but recently there has been an increasing interest in the biological function and therapeutic roles of natural products and their ecological role in regulating interactions between microbes. Mushrooms possess antibacterial and antifungal compounds to survive in their natural environment. It is therefore not surprising that antimicrobial compounds with more or less strong activities could be isolated from many mushrooms that they could be of benefit for human. In recent years, medicinal mushrooms have been re-investigated as sources of novel antibiotics and antivirulence mainly as a result of increasing difficulty and the cost of isolating novel bioactive compounds from the Actinomycetes and Streptomyces. (Mahendra Rai et al., 2005)

Pleurotus florida is one of the choice edible mushrooms which can be cultivated in many countries in the subtropical and temperate zones. *Pleurotus* is referred to as ‘oyster mushroom’ worldwide, and are rich sources of beta-glucan, phenolic compounds, dietary fibre, terpenes, saponins and flavonoids which exhibit immunomodulatry properties and combat bacteria, fungi and viruses.(Vamanu et al., 2012). These mushrooms have been used extensively in traditional medicine as antimicrobial,

Tele:

E-mail addresses: sumathysaravanan.31@gmail.com

antioxidant, anticancer and antitumor agents (Iwalokun et al., 2007). However, not much literature is available with regard to their anti-quorum sensing activity of *Pleurotus florida*. The present study is aimed to screen *P.florida* for its anti-QS property using *Pseudomonas aeuroginosa* as bioreporter strain.

Materials ad Methods

Sample Collection and Mushroom Extract Preparation

The fruit bodies of mushroom *Pleurotus florida* were obtained from Mushroom house, Department of Biology, Gandhigram Rural Institute – Deemed University, Gandhigram, Dindigul, Tamilnadu, South India. Fruit bodies of mushrooms were dried in a solar drier at 45 -50ºC for 48 h, the dried mushrooms were finely ground and kept in a freezer at -20ºC until use. The dried and powdered mushroom material was kept in an oven at 55^0C for 1hr before use.5g of powdered material was mixed with 50ml of methanol and chloroform separately in different beakers and then placed in the rotary shaker for 24hrs. The aqueous solutions were then filtered using Whatmann No.1 filter paper and concentrated in vacuum for 15min at 37^0C using a rotary evaporator (Nuran and Olcay, 2012).

Swarming Motility

Swarming motility assay was performed in modified Luria Bertani (LB) medium (Peptone – 5.0g, Beef Extract – 3.0g, agar 15.0g) supplemented with 0.5% glucose (Dereje et al., 2013). 300µg mushroom powder was added to the medium and the agar plate was allowed to solidify for one hour. 2µl overnight cell culture of *Pseudomonas aeuroginosa* maintained in LB broth was carefully spotted on the plate surface at the centre. Then the plate was incubated at 37^0C for 24hrs and the result was observed.

Inhibition of AHL Production

Two sets of six boiling tubes with 10ml of nutrient broth glucose medium were taken, in which added 10µl overnight bacterial culture. In one set of the six tubes five tubes were marked as treatments (T1-T5) which receives 0.1ml of methanol extract of mushroom at different concentration 100, 200, 300, 400 and 500µg and to the sixth tube added 0.1ml of methanol devoid of mushroom extract was taken which serve as the control. Similarly the other set of six tubes were made for chloroform extracts. The boiling tubes were incubated at 37^0C for 24hrs. After incubation the contents in the tubes were centrifuged at 10,000 rpm for 15min. 400µl of supernatant from centrifuged tube was taken in microfuge tube and 500µl of 1:1 mixture of hydroxyl amine (2M): NaOH (3.5M) was aliquoted and mixed with the content in all the tubes. Subsequently the same amount of 1:1 mixture of ferric chloride (10% in 4M HCl):95% ethanol was added. The quantities of lactones inhibited were determined spectrophotometrically at 540nm. (Uroz and Heinonsalo, 2008)

Inhibition of biofilm formation

Biofilm formation was assessed by the modified method of Chong et al. (2011), which quantify the adherence of bacterial cells to the wells of 96 well microtitre plates. Twelve wells, six wells in first row and another six wells in second row in the plates were selected and in each well 170µl of Luria Bertani broth (LB: 1.0% Tryptone, 0.5% yeast extract, 1.0% sodium chloride, pH 7.0), 20µl of overnight *P.aeuroginosa* culture were added . 10µl of mushroom extracts at different concentration 100, 200, 300, 400 and 500µg were added to the corresponding wells starting from 1^{st} well to the 5^{th} well, except the sixth well in both the rows. 10 µl of methanol was added in the sixth well in first row and 10 µl of chloroform added in the second row respectively serves as the control. Contents in the wells were mixed thoroughly and incubated at 37^0C for 24hrs. After incubation, the supernatants were removed and the plates were air dried. For fixation of the biofilm, 100µl of 99% methanol was added and incubated for 15min at room temperature. Then 100µl of crystal violet solution was added to all wells. After 20 min the excess crystal violet solution in the wells were removed by washing the plates under running tap water. The bound crystal violet was released by adding 150µl of 33% acetic acid. The absorbance was measured at 590nm using spectrophotometer.

Result and Discussion

Pleurotus florida, an oyster mushroom is primarily consumed for its nutritive value. In recent times its values its medicinal values are being realized. The present study revealed that this mushroom also has anti quorum sensing potential.

Study on swarming motility of the opportunistic pathogen *P.aeuroginosa* was used to investigate their flagellar motility which is a key component related to the multicellular behavior, biofilm development and AHL, autoinducer production of an organism causing quorum sensing. The results of the present study indicated that the *P.florida* mushroom extract exhibited reduction in swarming motility which confirms that this mushroom extract has anti-QS activity. Caiazza et al., (2007) and Heydron, (2002) also reported similar results with modified agar concentrations wherein *P.aeuroginosa* showed good swarming motility. Swarming motility inhibition was also reported from the organic solvent extracts of *Astragalus membranaceus* and *Mangolia officinalis* (Sandy Siew and Foong, 2012).

AHL production and Biofilm formation by the organism have profound effect on infection dynamics and pathogenesis. Biofilm formation by many pathogens is intimately linked with the formation of inter bacterial communication known as QS, in which small diffusible signaling molecules (AHLs) called autoinducers globally regulate gene expression. Using *P.aeuroginosa* as a bioreporter, anti-QS property of mushroom extract was clearly demonstrated. The present study revealed the inhibition of AHL production with different concentration of mushroom extracts. Percentage of inhibition ranged from 37.89-58.94% for methanol extract and 50.05-70.05% for chloroform extract, the inhibition of AHL production was concentration dependent. Compared to that of the methanol extract chloroform extract showed higher percentage of inhibition of AHL production (Fig-1). Givskov et al. (1996) in his study with *D.pulchra*, (the red algae known as the Australian sea weed) reported that they are efficient in controlling bacterial colonization by interfering with the AHL system.

Biofilms are complex systems that can be viewed both as microbial ecological communications and as mechanical objects. The present study implies the inhibition of biofilm formation by the mushroom extract and the percentage of inhibition is concentration dependent (Fig-2). In methanol extract the percentage of inhibition ranged from 33.9-83.9% and for chloroform extracts the percentage of inhibition ranged from 60.7-82.1%. Among the two extracts of mushroom tested, both the extracts showed appreciable inhibition, in which chloroform extract showed higher percentage of inhibition for AHL production and biofilm formation than methanol extract. Results obtained from the present study are in accordance with the extracts of *Ganoderma lucidum*, *Tremella fuciformis* (Zhu et al., 2011); and *Medicago truncatula* (Daniels et al., 2002), which exhibited similar inhibition on AHL production and biofilm formation. Uroz and Heinonsalo,(2008) also stated that the plant-root associated fungi such as *Phialocephala fortinii* and *Melinomyces variabilis* and ascomycetes isolates have been

found to have the ability to degrade the AHL and have been proposed as an option for diminishing the bacterial virulence. This study on *Pleurotus florida* indicated the potential of oyster mushroom as a source of anti-QS compounds and also highlight the importance of evaluating the unexplored diversity of mushrooms for this property.

Conclusion

Present research has proven that mushroom, *Pleurotus florida* has the ability to inhibit the signaling molecule produced by the bacteria *Pseudomonas aeuroginosa* and this will obstruct the bacteria virulence factors by disrupting their communication systems. Anti-QS potential of *Pleurotus florida* could offer an alternative mode of action against opportunistic pathogenic bacteria that use QS to regulate virulence expression. The findings high lights that there lies a rich source of medicinal properties still to be explored from mushroom, in addition that contain compounds able to inhibit QS and QS related virulence processes. Further study to identify the active compounds of the mushroom extract and mechanism of action of QS by the active compounds is underway.

Figure 1: Inhibition of AHL production (%)

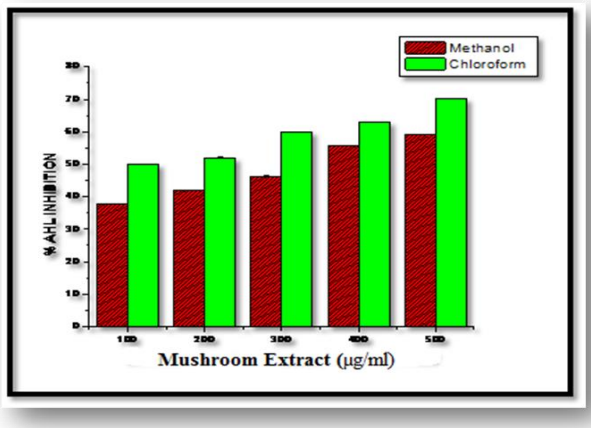
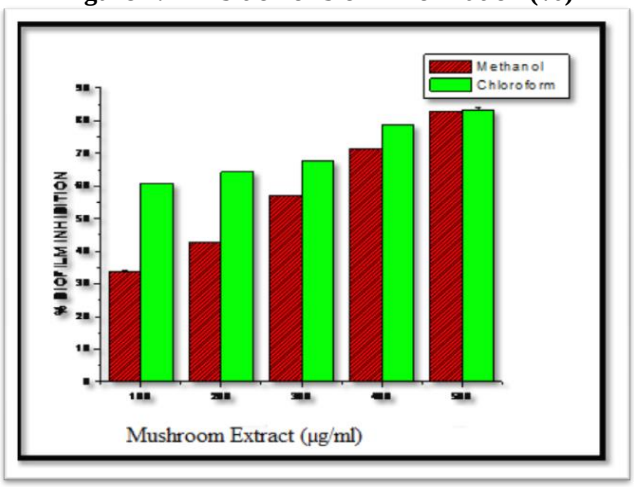


Figure 2: Inhibition of biofilm formation (%)



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