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OPTIMIZATION OF ABIOTIC FACTORS IN *Schizophyllum commune* TO DETERMINE ITS LIGNINOLYTIC ACTIVITIES AND ANTIBACTERIAL PROPERTIES

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DEDICATION

This thesis is dedicated to:
To my supervisor, Dr. Andrew Anak Ngadin, and my co-supervisor Ts. Dr. Fauziah binti Tufail Ahmad who convincingly guided and encouraged me to complete this study.

To my beloved parents, Prof. Dr. Rosnan bin Yaacob and Zamilah binti Idris for their constant prayers and support throughout my entire process.

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Main Supervisor : Andrew Anak Ngadin, PhD

Co-Supervisor : Ts. Fauziah Binti Tufail Ahmad, PhD

Faculty/Institute : Faculty of Fisheries and Food Science

Agricultural lignocellulosic waste is a potential source of serious environmental pollution. Studies on *Schizophyllum commune* Fr. as a lignocellulose degrader and its antibacterial properties, which are controlled by abiotic factors, can play an important role to better understand this species for the degradation of various lignocellulosic waste. In this study, the ligninolytic enzyme activities of *S. commune* and its antibacterial activities were investigated. The results showed that a higher level of mycelium formation of *S. commune* was observed in malt extract agar medium supplemented with yeast extract and glucose (MYGPA) at pH 5 and 28°C. The aeration factor significantly affected ($p < 0.05$) the growth, density of mycelium, and fruiting body formation of *S. commune*, based on oxygen and carbon dioxide consumption. Furthermore, eight substrates were investigated to compare their ability in producing higher activities among three ligninolytic enzymes under similar conditions. It was found that the maximum lignin peroxidase activity (1378.69 ± 41.76 U/ml) was obtained using coconut leaves as the subtrate , while manganese peroxidase showed its maximum activity (16.82 ± 4.36 U/ml) when using banana leaves, and laccase

(49.00 ± 18.35 U/ml) when using spiny bucida after eight days in solid state fermentation (SSF). The complex structures of different lignocellulose materials were found to be degraded differently by *S. commune* as observed through the scanning electron microscope (SEM). Meanwhile, *S. commune* antibacterial activities against food poisoning bacteria, namely two types of Gram-positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*) and three types of Gram-negative bacteria (*Escherichia coli*, *Salmonella* Typhimurium, and *Pseudomonas aeruginosa*) were investigated. The crude extract of *S. commune* was obtained from its cultivated fruiting body in rubber sawdust using different extraction solvents (100% methanol, 80% methanol, 80% acetone, 80% dichloromethane, and 80% ethyl acetate). *S. commune* 80% ethyl acetate extract was found to be the most effective in suppressing the growth of *S. aureus*. Furthermore, the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of this extract against *S. aureus* were 50 mg/ml and 100 mg/ml, respectively. The findings show that based on optimal environmental factors, *S. commune* has the ability to use agricultural waste lignocellulose as an alternative growth subtrate to rubber sawdust, which can benefit the proper waste management, while its antibacterial properties can be beneficial to the pharmaceutical industry.

Abstrak tesis yang dikemukakan kepada Senat Universiti Malaysia Terengganu sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

PENGOPTIMUMAN FAKTOR ABIOTIK DALAM *Schizophyllum commune* UNTUK MENENTUKAN AKTIVITI LIGNINOLITIKNYA DAN CIRI-CIRI ANTIBAKTERIA

NUR DIYANA BINTI ROSNAN

2021

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Pusat Pengajian : Fakulti Perikanan dan Sains Makanan

Sisa lignoselulosa daripada pertanian adalah berpontensi menjadi sumber pencemaran alam sekitar yang serius. Kajian tentang *Schizophyllum commune* Fr. sebagai pengurai lignoselulosa dan sifat antibakterianya yang dikawal oleh faktor abiotik, boleh memainkan peranan penting dalam memahami spesies ini dengan lebih baik untuk penguraian pelbagai sisa lignoselulosa. Dalam kajian ini, aktiviti enzim ligninolitik *S. commune* dan aktiviti antibakterinya telah disiasat. Hasil kajian menunjukkan bahawa tahap pembentukan miselium *S. commune* yang lebih tinggi diperhatikan pada medium agar ekstrak malt yang ditambah dengan ekstrak yis dan glukosa (MYGPA) pada pH 5 dan 28 °C. Faktor pengudaraan secara signifikan mempengaruhi ($p<0.05$) pertumbuhan, ketumpatan miselium, dan pembentukan badan buah *S. commune* berdasarkan penggunaan oksigen dan karbon dioksida. Selanjutnya, lapan substrat telah dikaji untuk membandingkan kemampuan *S. commune* dalam menghasilkan aktiviti enzim ligninolitik yang lebih tinggi pada keadaan yang sama. Didapati bahawa aktiviti lignin peroksidase yang maksimum (1378.69 ± 41.76 U/ml) diperoleh dengan menggunakan daun kelapa, sementara mangan peroksidase menunjukkan aktiviti

maksimumnya (16.82 ± 4.36 U/ml) ketika menggunakan daun pisang, dan lakase (49.00 ± 18.35 U/ml) semasa menggunakan pokok doa setelah lapan hari dalam penapaian berkeadaan pepejal (SSF). Struktur kompleks bahan lignoselulosa yang berbeza terdegradasi secara berbeza oleh *S. commune* seperti yang diperhatikan melalui pemerhatian mikroskopi elektron imbasan (SEM). Sementara itu, aktiviti antibakteria *S. commune* terhadap bakteria keracunan makanan, iaitu dua jenis bakteria Gram-positif (*Bacillus cereus* dan *Staphylococcus aureus*) dan tiga jenis bakteria Gram-negatif (*Escherichia coli*, *Salmonella* Typhimurium, dan *Pseudomonas aeruginosa*). Ekstrak mentah *S. commune* telah diperoleh dari badan buah yang dihasilkan dalam habuk papan getah dengan pelarut pengekstrakan yang berbeza (100% metanol, 80% metanol, 80% aseton, 80% diklorometana, dan 80% etil asetat). Ekstrak etil asetat *S. commune* 80% telah didapati paling berkesan dalam menghalang pertumbuhan bakteria patogen positif, *S. aureus*. Tambahan pula, kepekatan perencatan minimum (MIC) dan kepekatan minimum bakteria (MBC) ekstrak ini terhadap *S. aureus* masing-masing adalah 50 mg/ml dan 100 mg/ml. Kajian ini menunjukkan bahawa *S. commune* berpotensi menggunakan lignoselulosa sisa pertanian sebagai alternatif untuk substrat habuk papan getah dan dapat memberi manfaat kepada pengurusan sisa yang betul, sementara sifat antibakteria dapat bermanfaat bagi industri farmasi.