Pleurotus sajor-caju can be used to Synthesize Silver Nanoparticles with Anti-fungal Activity against *Candida albicans*.

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INTRODUCTION

Silver nanoparticles (AgNPs) can be synthesized from biological source. Thus, higher fungus such as mushroom, *Pleurotus sajor caju* has been used to synthesis AgNPs due to the bioactive compounds present in the extract such as vitamin, protein and polysaccharide^{1,2}. These compounds will serve as reducing agent and natural stabilizer to produced AgNPs from Ag⁺. Plus, *P. sajor caju* is also widely cultivated in Malaysia and the short production period makes it available throughout the year.

In fact, previous study showed that AgNPs has positive inhibition towards pathogenic yeast, *C. albicans*³. *C. albicans* is opportunist yeast and normally present in healthy people with commensalism interaction to the host. However, it can be virulent and cause severe infection in stress condition such as limited or absence of fermentable carbon source. The yeast is able to switch its phenotype from budding yeast to hyphae for cell penetration either forming biofilm on cells or escape from the macrophage⁴. Thus, in this condition, glyoxylate cycle was required to utilize the non-fermentable carbon sources to generate glucose for energy and cell function. Unfortunately, glyoxylate cycle was happened to be one of the factors contributed to the *C. albicans* virulence. Glyoxylate pathway is required in survival of *C. albicans* upon glucose depletion. It aids virulence of *C. albicans* through maintaining cell haemostasis and survival particularly during phagocytosis. This pathway is mediated by Isocitrate lyase (ICL) and malate synthase (MLS) for the synthesizes of oxaloacetate (C₄) for glucose production via gluconeogenesis pathway. Therefore, in this study, the effect of AgNPs on the enzyme responsible for glyoxylate cycles; isocitrate lyase (*ICL1*) may provide a brief understanding of the mechanism of AgNPs inhibition towards yeast at transcriptome and proteome level (Figure 1).

Figure 4. (a) FTIR spectrum of AgNPs and (b) X-ray diffraction pattern of AgNPs

2. Antifungal activity of AgNPs on *C. albicans*.

Figure 5 *C. albicans* colonies on YPL (yeast extract, peptone and lactate) agar medium (a) Growth of colonies was observed at different concentration and MIC and MFC value of AgNPs was 250 and 500 mg/L respectively. (b) different concentration of fluconazole (positive control) and MIC and MFC value was 1.96 and 3.91 mg/L respectively c) *C. albicans* in YPL served as control (triplicate)

3. Impacts of AgNPs on *C. albicans* *ICL1*.

Figure 1. Schematic diagram showing overview of study

1. Synthesize and Characterization of AgNPs.

Figure 2. (a) UV spectra of AgNPs synthesized from *P. sajor caju* extract at different time intervals (b) Pale yellow of *P. sajor caju* extract turned to reddish brown after 72 hours incubation with 1mM AgNO₃ thus indicate the formation of AgNPs.

Figure 3. TEM images of AgNPs in deionized water. The morphology of particles were in spherical shape with the average diameter size 11.68 nm and zeta potential, -8.54.

Figure 6. The effect of AgNPs on *Ca/ICL1* (a) *ICL1* gene was downregulated at 2 and 4 hours incubation then it was upregulated at 6 hours incubation (b) *ICL1* enzyme was increase at 2 and 6 hours incubation. Therefore, it shows that the AgNPs was able to regulate the expression of *Ca/ICL1* in 6 hours incubation with significant reduction at 2 and 4 hours incubation in gene and protein of *Ca/ICL1* respectively.

Conclusion

AgNPs was able to be synthesised from *P. sajor caju* extract. After 3 days of incubation, AgNPs was shown to have small particle diameter which was less in 100 nm. Thus, the small size and neutral zeta potential was suggested to be one of the factor in inhibit the growth of *C. albicans* in YPL medium. Furthermore, the AgNPs was shown to be able to regulate the expression of *C. albicans* gene and enzyme. The expression of *Ca/ICL1* and *Calcl1* was decrease after 2 hours addition of AgNPs and *C. albicans* was able to regulate the stress condition and increase the *Ca/ICL1* expression at following hours to survive and growth. However, the expression of *ICL1* enzyme in treated cells showed that it still lowered than *C. albicans* without AgNPs (untreated).

For future work, synthesis of AgNPs can be optimized to increase the potential of AgNPs as antifungal agent and the exact mechanism of inhibition can be determined by thorough analysis.

Acknowledgement

Thanks to Universiti Sains Malaysia for the funding of this study.

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