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Isolation and identification of glucose oxidase producing fungi from Nelumwewa and Maha Oya hot water springs, Sri Lanka

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Glucose oxidase has a wide range of industrial applications, and the main focus of this study was to isolate fungi with thermostable glucose oxidase activity from Sri Lankan hot water springs. Water samples collected from Nelumwewa and Maha Oya hot water springs in the Polonnaruwa district were subjected to serial dilution and inoculated on a medium contained peptone, glucose, agar, and chloramphenicol, and incubated at 30^{o}C for 6 days. In total, six fungal colonies were isolated and screened for glucose oxidase production, which was done using two different media. Screening medium I consisted of glucose, peptone, o-dianisidine, horseradish peroxidase and agar. Enzyme production was indicated by the appearance of brown color ring around the colony. Screening medium II consists of two layers. The bottom layer contained glucose, peptone, $CaCO_3$ and agar, and the top layer contained glucose, KI, starch and agar. Wells were created on top layer and isolated fungi colonies were inoculated into the wells. Upon enzyme production, a blue/violet color appeared on the top layer around the colony. Fungus colonies showing glucose oxidase production were grown on slants containing peptone, glucose, and agar and incubated at 30^{o}C for 14 days until sporulation. Slants were washed with sterile water and pooled. Submerged fermentation was done in a medium containing $NaNO_3$, KH_2PO_4, $MgSO_4.7H_2O$, $FeSO_4.7H_2O$, $CaCO_3$, peptone, and glucose. Spore suspension and fermentation medium was used in 1:19 ratio for submerged fermentation. It was incubated at 30^{o}C for 3 days on a rotary shaker and the activity obtained was 0.85U/mL. The fungus having highest glucose oxidase production was identified morphologically, as well as by 18s rRNA sequencing, as *Talaromyces funiculosus* (Genbank: KT148636.1) and catalogued as *Talaromyces funiculosus* AMS1. All media used in this study has the pH of 5.6±0.2 and Sigma Aldrich standard glucose oxidase assay was used in enzyme activity calculation. Further studies are in progress to investigate the industrial applicability of glucose oxidase obtained from the fungus *Talaromyces funiculosus* AMS1.

Keywords: Glucose oxidase, *Talaromyces funiculosus AMS1.*

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