

Supplementary material

Sophorolipid exhibits antifungal activity by ROS mediated endoplasmic reticulum stress and mitochondrial dysfunction pathways in *Candida albicans*

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Production extraction, purification and characterization of sophorolipid

Production, extraction and purification of SL were carried out as reported by Haque et al. Briefly, *Starmerella bombicola* MTCC 1910 was grown in a 100 ml Erlenmeyer flask containing 20 ml of YPD medium, [yeast extract (10 g/L), peptone (20 g/L) and dextrose (20 g/L)] as seed culture. The production medium contained glucose (100 g/L), malt extract (10 g/L), urea (1 g/L), and cotton seed oil (100 g/L). Around 2% of the seed culture was inoculated in the SL production medium and incubated in an orbital shaker for 7-8 days at 30°C and 200 rpm.

The SL was separated from the broth by ethyl acetate extraction and concentrated at 40 °C by vacuum evaporation. The residual hydrophobic components were washed with n-hexanes to obtain crude SL. This crude mixture of SL was subjected to HPLC (Shimadzu) analysis fitted with UV detector (207 nm) and an RP-C18 column (Merck, 5 µ, 4.5× 250 mM) using gradient elution. Initially, acetonitrile:water (30:70) was used for 5min gradually the composition of the eluting liquid was changed to acetonitrile: water:: 80:20 in 25 min and maintained there for next 25 min. The flow rate was kept at 0.5 mL/min and injection volume was 10 µL. Column chromatography was carried out to isolate the lactonic SL. Briefly, around 50 g of silica mesh size (60–120) in hexane was packed in (50× 5 cm) glass column. Around 200 mL of eluent (chloroform/methanol) was run through the column before loading the sample. Approximately, 300–400 mg of crude SL dissolved in a small volume of ethanol was mixed with silica (3.5 gm) and evaporated under reduced pressure at 40 °C. Once the silica was fully dried into a free-flowing powder, it was loaded into the column. Diacetylated form of lactonic SL was eluted from the column by using chloroform and methanol at a ratio of 98:2 and dried under vacuum at 40 °C and stored for further use. HPLC analysis showed a single peak of SL with more than 99% purity. Different functional groups present in the sample were identified by FT-IR spectroscopy (Bruker optics, vortex 70) confirming the

presence of lactonic form of SL in the sample. This preparation of SL was used for determining the anticandida activity in subsequent experiments.

Reference:

1. F. Haque, M. Alfatah, K. Ganesan and M. S. Bhattacharyya, *Sci. Rep.*, 2016, **6**, 23575.