Fungal DNA Extraction

The fungal mycelium, weighing 2 g, was crushed and ground into a fine powder using liquid nitrogen (LN_2) in a sterilized mortar and pestle. The resulting powder was then transferred to a 1.5 ml Eppendorf tube. To this tube, 650 µl of Cetyl trimethyl ammonium bromide (CTAB) buffer and 150 µl of β-mercaptoethanol (Thermos Fisher Scientific, USA) were added. The tube was incubated at 65°C for 1 h in a water bath, followed by centrifugation at 12,000 rpm for 10 min. After collecting the supernatant, an equal volume of phenol: chloroform: isoamyl alcohol (PCI) in the ratio of 25:24:1 was added. Centrifugation was repeated at 12,000 rpm for 20 min. Approximately, 500 µl of the supernatant was collected and mixed with 500 µl of isopropanol and 50 µl of sodium acetate. The tube was incubated overnight at 4 °C, followed by centrifugation at 10,000 rpm for 10 min at 4 °C. The resulting DNA pellet was washed with 70% ethanol (500 µl) and centrifuged at 10,000 rpm for 10 min at 4 °C. After discarding the ethanol, the DNA pellet was air-dried for approximately 20 min in the laminar airflow.

The extracted DNA was resuspended in 50 µl of 10 mM Tris-EDTA (pH 8), and its quantification was determined using a UV-Vis spectrophotometer Nanodrop (Thermo Fisher Scientific, NanoDrop 2000, USA), by observing the 260/280 nm ratio. The purity of the DNA was further confirmed by agarose gel electrophoresis.

Composition of cetyl trimethyl ammonium bromide (CTAB) buffer for 100 ml

Materials/Ingredient	**Quantity**
CTAB (2%)	2.00 g
0.5 M Ethylene diamine tetra acetic acid (EDTA)	10.00 ml
5.0 M NaCl	28.00 ml
1.0 M Tris HCl, pH 8.0	10.00 ml
β-Mercaptoethanol	200 µl

PCR Amplication and PCR conditions

The amplification of the internal transcribed spacer (ITS) gene, was performed using the ITS1 and ITS4 primers (White *et al*., 1990). A PCR reaction mixture of 25 µl was prepared by adding 12.5 µl of master mix, 1 µl of forward and reverse primer each, 1 µl of template, and 9.5 µl of nuclease-free water. The PCR reactions underwent an initial denaturation step of 5 min at 95 °C, followed by 35 cycles consisting of denaturation of 1.5 min at 94°C, annealing of 2 min at 55°C, and extension of 3 min at 72°C. A final extension step of 5 min at 72°C was performed using a thermal cycler (Applied Biosystems™ ProFlex™ PCR System). The resulting PCR product was analyzed by agarose gel electrophoresis using a 1% gel and ethidium bromide as the intercalating dye.