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Rapid and Efficient Procedure for Genomic DNA Extraction from *Trichoderma* spp.

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Molecular Biology experiments including gene cloning, restriction digestion, identification by 18S r-DNA requires purified and high quality genomic DNA. The major problems in DNA isolation from fungus are impurities such as polysaccharides, protein and RNAs which interfere in PCR reaction. In this study, proficient method for DNA extraction without using liquid nitrogen, CTAB or lysozyme was optimized. The method utilizes very few chemical compounds. The method involved crushing of fungal mycelia in lysis buffer containing SDS, incubation at 65ºC, extraction by chloroform, phenol and isoamyl alcohol and finally DNA precipitation by cold ethanol. The results showed DNA with high yield which can be utilized for PCR purposes.

Introduction

Trichoderma spp. enhances plant growth and productivity in several agricultural crops is important for the control of other fungal diseases such as soil and seed borne (Vazquez-Angulo *et al.*, 2012). The use of *Trichoderma* spp. in the field can help to reduce the application of chemical pesticides and conserve the soil and its ecosystem. Thus *Trichoderma* cultures were isolated from soil of Yavatmal, Vidarbha region of Maharashtra to be used as local and adapted species for different crop plants. Identification of diverse species of *Trichoderma* isolated from soil is an important issue. Previously microorganisms were identified at species level by means of the application of the concept of Morphological species recognition i.e. MSR in combination with its other phonotypic traits. The visual identifications become highly error prone due to lack of well defined morphological characteristics in cultures. So to limit the above drawbacks DNA based characterization of isolates may reflects the clear picture of relationships than do morphological characters. Purified and high concentration of genomic DNA is a perquisite for taxonomic studies based on molecular characterization. Various authors described different methods for DNA isolation from *Trichoderma* (Gadambe *et al.*, 2018; Vazquez-Angulo *et al.*, 2012; Cassago *et al.*, 2002). The major challenges for

isolation of fungal DNA of good quality lies in breaking of rigid cell wall, high polysaccharide content and fungal nuclease (Fredrick *et al*.2005). All these methods include use of CTAB, proteinase K (Wilson 1990), lysozyme (Flamm *et al.,* 1984), high speed cell disruption (Muller, 1998) and liquid nitrogen (Lee *et al*., 1988).

The method described here for extracting genomic DNA from filamentous fungi did not use liquid nitrogen, CTAB or lysozyme for cell wall lysis and yielded DNA of high concentration without much affecting quality and purity. In this method we utilized 3% SDS in lysis buffer and fungal mycelia were crushed in mortar and pestle without using liquid nitrogen. The DNA was extracted using solvent extraction method using phenol, chloroform, isoamyl alcohol and extracted in TE buffer.

Materials and Methods

Cultures and growth conditions

Six isolates of *Trichoderma* spp were isolated from the soil samples collected from different locations of Yavatmal, Maharashtra, India by serial dilution technique. Different isolates were grown on Potato dextrose broth (Table 1) and incubated for 3-4 days at 25 ±2 ºC over incubator shaker at 120 rpm.

DNA extraction

Fungal mycelia of 250 mg from six different isolates of *Trichoderma* spp. were crushed in 500µl of lysis buffer (50mM Tris HCL, 50mM EDTA (pH 8.2), 3% SDS) in mortar and pestle individually. After crushing macerate was transferred to 2ml labeled autoclaved micro centrifuge tube separately. 1ml of lysis buffer was added again in each tube and vortexed it for 2-3min and incubated at 65ºC for 1 hour in water bath. 500 µl of Chloroform: phenol (1:1) was added to each tube and vortex briefly. Tubes were centrifuged at 14000 rpm for 10 min at 4°C using cooling centrifuge (Eppendorf, Germany). The supernatant (aqueous layer) was collected in fresh labeled 1.5ml micro centrifuge tube with the help of micro pipette and 500 µl of chloroform: isoamyl alcohol (24:1) was added to each samples. The tubes were inverted several times slowly and then centrifuge at 14000 rpm for 5min at 4°C. Two distinct phases were observed from which supernatant was transferred in fresh autoclaved micro centrifuge tube without disturbing middle layer. Again 500 µl of chloroform: Isoamyl alcohol (24:1) was added to the tubes and inverted several times and then centrifuge it at 14000 rpm for 5 min at 4°C. The aqueous phase was transferred to fresh 1.5 ml micro centrifuge tube and ice chilled 100% (absolute) ethanol was added and inverted the tubes gently for 2-3 min. The tubes were incubated for 30 min at -20ºC for DNA precipitation. The micro centrifuge tubes were centrifuge at 14000 rpm for 5 min at 4°C and supernatant was discarded after centrifugation. 500 µl of 70% chilled ethanol was added to the pellet of each tube and the tubes were inverted gently 4-6 times to wash the DNA pellet.

The tubes were again centrifuged at 14000 rpm for 3 min at 4°C and the supernatant was discarded with the help of micropipette. The tubes were then transferred to Vacuum evaporator (Eppendorf, Germany) and vacuum evaporated the ethanol for 2-5 mins depending on the amount of ethanol present in the microcentrifuge tube. 100 µl of TE buffer (10 mM Tris base and 1 mM EDTA, pH 8.4) was added to dissolve/resuspend the DNA. The tubes were incubated overnight at 37 ºC for complete resuspension and store at -20 ºC. The yield and purity of DNA was measured at 260, 280 and 230 nm.

Results and Discussion

The isolated genomic DNA was quantified by using spectrophotometric measurement (Eppendorf, Germany) at 260 and 280nm. The yield and purity of isolated DNA for six *Trichoderma* isolates in triplicate were noted in Table 2.

The present DNA extraction method yielded good quantity and quality of pure DNA. This DNA can be use for different applications. DNA data reflect the genotype of the organism and may give a clearer picture of relationships than do morphological characters. Polymorphism at the DNA level can be studied by several means, the most common of which is the analysis of restriction fragment length polymorphisms (RFLP), Amplified fragment length polymorphism (AFLP), Random amplified polymorphic DNA (RAPD), simple sequence repeats (SSR), etc. This method did not require liquid nitrogen (It can be difficult to procure in remote locations) or magnetic beads (tissue leaser) to crush the sample which is mostly unavailable to some undergraduate laboratories.

Table.1 Composition of potato dextrose broth

Composition	g/l
Potato infusion	200
Dextrose	20
Distilled water	1000ml
pH	5.6±0.2

Table.2 Concentration and purity of genomic DNA isolated from six different isolates of *Trichoderma* spp.

Isolates of *Trichoderma*		Concentration of DNA ($ng.\mu l^{-1}$)	Purity (260/280 ratio)
T1	T1-1	852.8	2.01
	T1-2	797.3	1.50
	T1-3	703.1	2.12
T2	T2-1	270.4	1.52
	T2-2	137.7	1.73
	T2-3	340.0	1.58
T3	T3-1	904.5	2.02
	T3-2	760.7	2.16
	T3-3	442.9	2.01
T4	T4-1	234.1	1.60
	T4-2	522.6	1.16
	T4-3	335.9	1.86
T5	T5-1	903.0	2.13
	T5-2	625.4	2.28
	T5-3	162.6	1.67
T6	T6-1	157.9	2.10
	T6-2	362.3	1.12
	T6-3	425.1	1.15

This method only required very simple instruments such as mortar and pestle, centrifuge machine and water bath. Certain hazardous chemicals like β-mercaptoethanol was also not used in our protocol and quality of DNA was also not got affected. This method is

practicable for undergraduate students at laboratory level.

Problems in fungal DNA isolation are polysaccharids, protein and RNAs impurities which further interfere in PCR amplification. The purpose of this study was to simplify and improve the currently available genomic DNA extraction method from filamentous fungi (Al-Samarrari and Schmidt 2000; Haugland *et al.*, 1999). The isolated DNA amount and quality obtained by this method were suitable for the PCR amplification, restriction digestion and other molecular analysis. This method has several advantages such as no requirement of liquid nitrogen, low instrumentation requirement, the number of steps in extraction procedure is minimal, the initial material i.e. sample requirement is less and small amount of chemicals required. This method is comparatively simple and rapid method of fungal DNA extraction.

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