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A RAPID AND SIMPLE METHOD FOR THE ISOLATION OF TOTAL DNA FROM *Trichoderma* spp.

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Abstract

DNA isolation from filamentous fungi is often tedious, time consuming and difficult because they have cell walls that are relatively unsusceptible to lysis. Here described a rapid, inexpensive and reproducible protocol for the isolation of DNA from the filamentous fungus, Trichoderma spp. The present protocol is based on the sodium dodecyl sulfate method without using β- mercaptoethanol and devoid of liquid nitrogen for the maceration. The precipitation of DNA was done using isopropanol and ethanol. The $A_{260/280}$ absorbance ratio of isolated DNA was ≈1.9 indicating that DNA fraction was pure and could be used for further analysis. The result of DNA electrophoresis in 0.8% agarose gel showed sharp clear bands of DNA, indicated the good quality of DNA. Since this protocol yielded the genetic DNA in sufficient quality and quantity this can be used for the successful isolation of DNA from Trichoderma spp.

Keywords- Trichoderma, β- mercaptoethanol, DNA isolation, SDS, Tris – HCl, lysis

I. INTRODUCTION

Molecular techniques are important analytical tools for the characterization and diagnosis of microbial population. DNA extraction is the primary and basic step of all these techniques. Therefore, these procedures must provide DNA in sufficient quantity and purity for further analysis [1]. Isolation of genomic DNA of fungi is laborious, expensive and time consuming.

The objective of the study was to develop an easy and rapid method for the extraction of fungal DNA from the bioagent, *Trichoderma* spp.

II. MATERIALS AND METHODS

2.1. Preparation of fungal culture

The present investigation was carried out for the isolation of total DNA from two isolates of *Trichoderma* spp. Isolated from the soil samples collected from Kerala. These two *Trichoderma* spp. *Viz., T. erinaceum* and *T. asperellum* were grown on potato dextrose broth and incubated at 28°C for a period of 3 days. The 83ycelia growth over the medium was collected in 2ml eppendorf tubes for further use.

2.2. DNA extraction buffer and solutions

The tubes and glass wares used for the procedure were autoclaved at 121°C for 20min, and then dried at 100°C before use. The tips used for the DNA extraction were DNase free and Rnase free. The extraction buffer was 2% SDS (w/v) containing 50mM EDTA (pH 8.0), 100mM NaCl and 250mM hydroxymethyl hydrochloride (Tris – HCl, pH 8.0). Additionally, a photosensitive solution of water saturated phenol (1:1 v/v) and a mixture of phenol: chloroform: isoamyl alcohol (25:24:1 v/v) were also prepared.

2.3. Genomic DNA extraction

The DNA extraction procedure was standardized and validated with modifications of the methods of Chakraborty *et al.,* 2010 [2]. A 250µl DNA extraction buffer was added to 300mg of fungal mycelium collected in 2ml eppendorf tubes and were shaken vigorously. The eppendorf tubes with the 84ycelia suspension were further incubated for 1 hr at 60°C with intermittent shaking. After incubation, the mixture was cooled to room temperature and centrifuged at 12,000 rpm for 15min. The supernatant was then transferred to another tube and added equal volume of water saturated phenol and further centrifuged at 12,000 rpm for 10 min. The aqueous phase was separated to a new tube and was further extracted with equal volume of phenol: chloroform: isoamyl alcohol followed by a centrifugation at 12,000 rpm for 15 min. The aqueous phase was then transferred to a fresh tube and an equal volume of ice cold isopropanol was added and the contents were incubated at -20°C for 30 min. for precipitating the total DNA. The DNA was pelletized by centrifugation at 12,000 rpm for 15 min. The DNA pellet was further purified by washing in 70 % ethanol by centrifugation at 5000 rpm for 5 min. The pellets were air dried and suspended in 200µl DEPC (Diethyl pyrocarbonate) – treated MiniQuantum (deionized) water and stored at -80°C until further use.

2.4. Qualitative and Quantitative estimation of DNA

Concentration, yield and quality control indices based on absorbance readings at 260 and 280 nm ($A_{260/280}$ ratio) were carried out with 2µl resuspended total DNA using a nano spectrophotometer. Ten microliters of total DNA solution was loaded on a 0.8% agarose gel, and electrophoresed to separate DNA.

III. RESULTS AND DISCUSSION

The method presented in this paper helps to eliminate much of the laborious and time consuming steps for the isolation of total DNA [3, 4, 5]. The DNA was extracted rapidly from the mycelium. The protocols including mechanical grinding with liquid nitrogen for the lysis of fungal cell wall was tedious, time consuming and more often yields poor results. This procedure is very handy since the quantity of SDS used for the lysis of fungal cellwall was very less. The steps including the addition of isopropanol and ethanol effectively precipitates the DNA, rendering it more stable.

With this protocol we obtained high yield of good quality genomic DNA from both the fungal samples. The absorbance ratio, $A_{260/280}$ was determined using nanodrop for evaluating the quality, quantity and integrity of the DNA. In both the cases, $A_{260/280}$ ratio was ≈1.9 indicating that DNA fraction was pure and could be used for further analysis (Table.1). The results of DNA electrophoresis in 0.8% agarose gel are demonstrated in Fig.1. The DNA produced clear sharp bands indicating the good quality of DNA.

Table 1. Absorbance ratio and yield of total DNA isolated from *Trichoderma* spp.

***Trichoderma* spp.**	**$A_{260/280}$**	**Yield (ng/ µl)**
T. erinaceum	1.99	278.1
T. asperellum	1.90	395.7

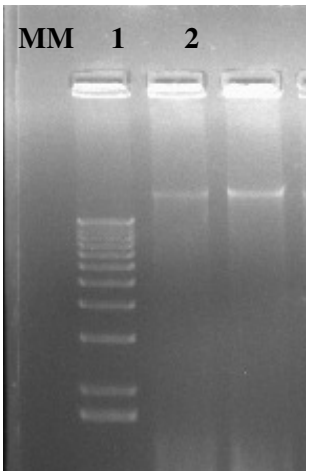

Fig. 1. Gel electrophoresis of the total DNA extracted from Trichoderma spp. MM : DNA marker (1Kb), lane 1: *T. erinaceum*, and lane 2: *T. asperellum*

IV. CONCLUSION

The present procedure of isolation of fungal DNA eliminates the step of mechanical shearing with the liquid nitrogen which is a tedious and time consuming step. In addition, one of the advantages of this protocol is that the omission of maceration reduces sample shearing and minimizes the risk of contamination. Hence this is a rapid, reliable and low cost technology for the extraction of DNA from fungal mycelium.

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