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Genomic DNA Isolation and Construction of DNA Libraries from *Trichoderma harzianum* and *Rhizopus* sp

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Abstract

This paper discusses rapid and reliable methods of extracting genomic DNA for construction of genomic DNA libraries from fungi: *Trichoderma harzianum* (E-58 strain) and *Rhizopus* species. Two methods for extraction of genomic DNA were compared. Both methods yielded comparable results. For the construction of genomic DNA libraries, the DNA was digested with *Bam*HI and ligated into pUC18. The plasmids were transformed into *E. coli*.

Key words: DNA isolation, Genomic, DNA libraries, *T. harzianum*, *Rhizopus* Sp.

Introduction

Microbial production of enzymes is preferred over plants and animal sources because of easier availability, structural stability and ease of genetic manipulation (Bilgrami and Pandey, 1992). Hemicellulolytic microorganisms play a significant role in nature by recycling hemicelluloses, one of the main components of plant polysaccharides (Kulkarni *et al*., 1999). Different fungal species have a great medicinal importance (Cohen *et al*., 1999). *Trichoderma harzianum* and *Rhizopus* sp. are preferred microorganisms for the production of industrially important enzymes like cellulases and xylanases. Strains of *Trichoderma* have been shown to secrete large amounts of efficient degrading enzymes (Wong and Saddler, 1992). Xylanases have been purified from different strain of *T. harzianum* (Ximenes *et al*., 1996; Silveria *et al*., 1999). *Rhizopus* sp. is also well known for the production of industrially important enzymes. Horicuhi *et al*. (1988) constructed genomic DNA library from *Rhizopus niveus* for the isolation of proteinase gene. Similarly different scientists have worked extensively on filamentous fungi such as *Rhizopus niveus*, *Rhizopus delimar*, *Rhizopus oryzae* (Houriuch *et al*., 1995, Hakhi and Akhasya 2001; Skorry, 2002).

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Hemicellulases are a diverse group of enzymes that hydrolyze hemicellulose. These enzymes have many biotechnological applications and their isolation from genomic DNA libraries is a subject of intense research. The biotechnological potential of these enzymes from various microorganisms has drawn a great deal of attention of researchers worldwide (Winters *et al*., 1996; Walker and Wilson, 1991). The introduction of recombinant DNA technology has resulted in the selection of xylanolytic and cellulolytic enzymes that are more stable for industrial applications (Sunna and Antranika, 1997). The genes of industrially important enzymes have been isolated from genomic DNA libraries of different fungal species (Basaran *et al*., 2001; Liang and Ljundahl, 1994).

In this paper we report the isolation and characterization of DNA and construction of genomic DNA libraries from *T. harziaunm* and *Rhizopus* sp.

Materials and Methods

Microorganisms

The test organisms used in these experiments were *Trichoderma harzianum* E-58 strain and *Rhizopus* sp. that were acquired from Molecular Biochemistry Lab, Dept of Chemistry and Department of Veterinary Microbiology, University of Agriculture Faisalabad, Pakistan, respectively.

Media and Culture Conditions

Trichoderma harzianum was maintained on agar slants (MYJ) containing (g L^{-1}) malt extract 5; yeast extract 2.5; glucose 10; agar 20. Freshly inoculated slants were incubated at 28^0 C for 5 days and stored at 4^0C. *T. harzianum* was cultivated in Vogel's medium [containing 0.5% Trisodium citrate, 0.5 % KH_2PO_4, 0.2% NH_4NO_3, 0.4% $(NH_4)_2SO_4$, 0.02% $MgSO_4$, 0.1 % peptone, 0.2 % yeast extract, pH 5.50] (Ahmed *et al*., 2003). The fungus was grown for DNA extraction according to Aslam *et al*. (2004).

Rhizopus sp was maintained on Sabouraud's dextrose agar medium [Containing g/100 mL peptone, 1;dextrose 4; agar, 3] (Awan and Rehman 2002).

DNA Isolation

Genomic DNA from *T. harzianum* and *Rhizopus* sp. were extracted by two methods i.e. by Lysis buffer

method and Extraction buffer method (Yamada *et al*., 2002; Chowe and Kafer, 2003).

(a) Lysis Buffer Method

Frozen mycelia of fungi were treated with lysis buffer (200 mM Tris-HCl pH 7.5, 0.5% SDS, 30 mM EDTA). Two hundred µL of 2.5 M potassium acetate was added and mixture was incubated and centrifuged. The sample were extracted with phenol/ chloroform/ Isoamyl alcohol and precipitated with one volume of cold isopropanol.

(b) Extraction Buffer Method

Five hundred µL of Tris-EDTA buffer was added to 0.2 g mycelia and centrifuged. Supernatants were extracted with 300 µL extraction buffer (200 mM Tris-HCl, 250 mM NaCl, 25 mM EDTA, 0.5% SDS) and 150 µL of 3 M sodium acetate was added. The subsequent steps were same as described for the lysis buffer method.

Characterization of Genomic DNA

Spectral characterization of genomic DNA was done. Hyperchromic effect was also observed. Tm and % GC were calculated according to Mandel and Marmur (1968). Percent GC was calculated by the following formula:

$$\%GC = 2.24(Tm - 53.9^{o}C).$$

Genomic DNA Libraries Preparations

Genomic libraries from *T. harzianum* and *Rhizopus* sp. were prepared using pUC18 as cloning vector and *E.coli* 10b as host. *E. coli* transformations were carried out according to standard procedures (Sambrook and Russell, 2001). Genomic DNA of *T. harzianum* and *Rhizopus* sp. was restricted with *Bam*HI and ligated into pUC18 plasmid linearized with *Bam*HI (Qurrat-ul-Ain *et al*., 2003).The genomic DNA fragments so ligated were transformed into *E. coli* (10b) by heat shock method (Ausubel *et al*., 1988) and plated on agar-ampicillin plates.

Results and Discussion

Isolation of genomic DNA

To select an optimal means of quick and reliable extraction of genomic DNA for construction of genomic DNA libraries, we used two methods for DNA isolation from the fungi.

DNA was isolated by lysis buffer method (Yamada *et al*.,2002) and extraction buffer method (Chow and Kafer, 2003) from mycelia of *T. harzianum* and *Rhizopus* sp grown in Vogel's medium and *Rhizopus* sp.

Both methods yielded good concentrations of DNA (Table 1). This was confirmed by running 0.8% agarose gel (Fig 1). Extraction buffer method yielded higher concentration of DNA as compared to lysis buffer method for *T. harzianum*.

Table 1: Comparison of DNA yields (mg/mL ±SD) of two extraction methods

Method	*Rhizopus* sp	*Trichoderma harzianum*
Lysis buffer Method	0.64± 0.186	0.67±0.186
Extraction buffer method	0.55±0.165	1.07±0.47

Earlier, Pinner *et al*. (1996) described a method for the isolation of genomic DNA from fungi, based on the spheroplastis preparation but this technique could not be used for the genomic DNA isolation due to it's requirements of large amount of cells and cost. Boiling method was found to be less suitable for genomic DNA isolation from different fungi (Biswas *et al*., 2001).

Franzot *et al*. (1997) compared different genomic DNA isolation methods. They found DNA yield obtained depends on isolation method and fungal species used. Total DNA extraction method used for construction of genomic DNA library was reported by Birger *et al.* (2003). They observed that the method in which they used ground-frozen mycelium in liquid nitrogen, was highly reproducible and resulted over 70 % recovery of good quality DNA.

In present study we have found both methods suitable for DNA isolation which are in agreement with Van *et al*. (1998) who reported that lysis buffer method is productive for extraction of genomic DNA from filamentous fungi, and Yamada *et al*. (2002) who found extraction buffer method suitable for isolation of mt DNA from yeast.

T_m of *Trichoderma harzianum* genomic DNA

T_m of *T. harzianum* genomic DNA was observed by treating genomic DNA at various temperatures. Control sample was allowed to cool at room temperature. T_m of *T.harzianum* genomic DNA was found to be 89^{o}C and % GC was calculated as 85.64% (Fig 2).

T_m of *Rhizopus sp.* genomic DNA

T_m of *Rhizopus sp.* genomic DNA was observed by treating genomic DNA samples at various temperatures. Control sample was allowed to cool at room temperature. T_m of *Rhizopus sp.* genomic DNA was found to be 87^{0}C and % GC was calculated 81.86% (Fig 3).

Construction of *T. harzianum* and *Rhizopus* sp. genomic DNA libraries

*Bam*HI digested genomic DNA fragments from *T. harzianum* and *Rhizopus* sp. (Fig. 4) were ligated into the pUC18 vector and the ligation mixture was then transformed into *E.coli* competent cells. Colonies from successful transformations were picked up and grown. Ligation of genomic DNA in pUC18 was confirmed through miniprep. Earlier, several workers have isolated genomic DNA from various

microorganisms and constructed genomic DNA libraries. Basaran *et al*. (2001) prepared genomic DNA library of *Pichia stiptis* by using pUC18 as cloning vector and *E.coli* DH5α as cloning host. Bashir *et al*. (1989) ligated DNA fragments of *Cellulomonas fimi* obtained through restriction with *Bam*HI, *Sau3* and *Sal1* into pUC18 linearized with *Bam*HI and *SalI.* The amplified DNA fragment was digested with *BglII* and *EcoRI* and ligated to pBLGI predigested with the same restriction enzymes by La Grange *et al*. (1996). Chauthaiwale and Deshpande (1992) constructed a complete genomic DNA library of *Chiania* in gt10 vector and scanned it for xylanase gene. Gibbs *et al*. (1995) constructed a genomic DNA library by ligating DNA fragments isolated from *Dictyoglomous thermophilum* strain Rt 46.B.1 in *ZapII* vector.

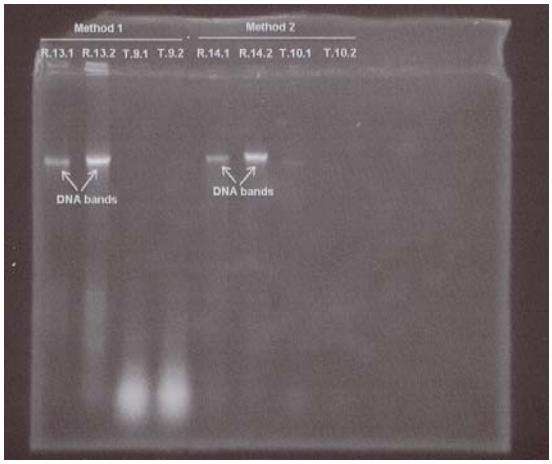


Fig. 1: Comparison of Lysis Buffer (method 1) and Extraction Buffer (method 2) for isolation of DNA from *Trichoderma harzinaum* and *Rhizopus* sp.

Fig. 2: Melting temperature of *Trichoderma harzianum* genomic DNA

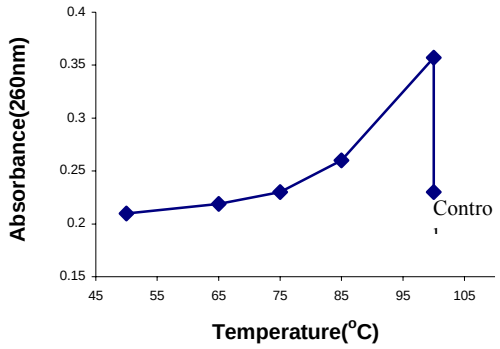
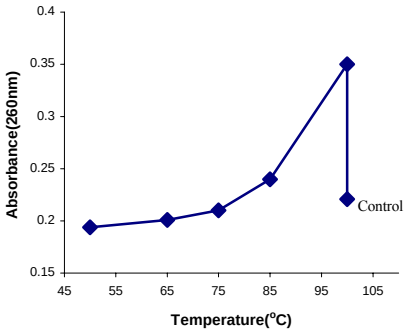


Fig. 3: Melting temperature of *Rhizopus sp.* genomic DNA



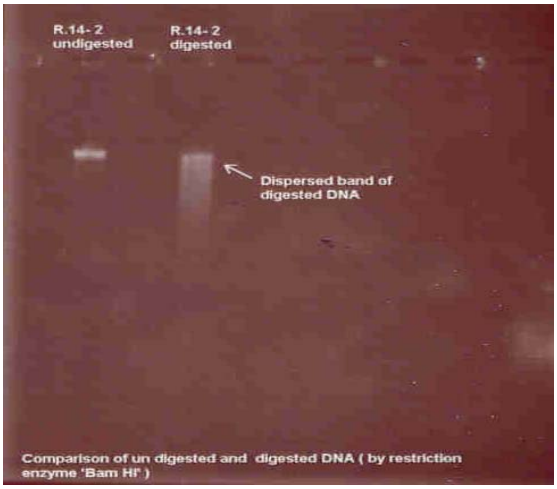


Fig. 4: *Bam* HI digestion of genomic DNA of fungi for the construction of genomic DNA libraries

Conclusion

Genomic DNA of *T. harzianum* and *Rhizopus* were successfully ligated into pUC18 vector and transformed into *E. coli*. This can be further utilized for screening of different genes present in these fungi.

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