Two simple non-enzymatic procedures to isolate high molecular weight DNA from fungi

Isolation of high molecular weight DNA is essential for molecular biology and recombinant DNA experiments. Various methods have been developed to isolate DNAs from yeasts and molds[1–5]. These procedures normally make use of cell wall degrading enzymes such as Novozyme, Glusulase, Helicase, Funcilase, Zymolase, etc., to degrade the cell wall of the fungi and proteases such as pronase or proteinase K to degrade the proteins after the cell lysis. In this communication we report two methods to isolate high molecular weight DNA from fungi without the use of enzymes One of the procedures, CTAB, is based on the method used for isolation of DNA from plant sources[6] with a slight modification wherein polyvinyl pyrrolidone (PVP) is omitted from the procedure as fungi do not have interfering amounts of phenolic compounds. In the first step CTAB is used to precipitate the carbohydrates at high temperatures and high salt concentrations, and in the subsequent step CTAB is used to precipitate the nucleic acids at low salt concentrations and low temperatures In the second method (TENS), relatively high concentrations of EDTA and SDS are used to inactivate nucleases after grinding the cells with acid-washed sand. Though both the methods yielded high molecular weight DNA from a thermophilic fungus, *Thermomyces lanuginosus*, the TENS method yielded 10–15 times more DNA per gram than the CTAB method (Table 1). Furthermore, a large amount of high molecular weight RNA was also observed in the TENS procedures (Figure 1). The DNA samples isolated using these procedures were readily digested with restriction enzymes such as PstI and Sau3A1 (Figure 2). Moreover, these procedures yielded high molecular weight DNA from other fungi such as *Saccharomyces cerevisiae* and *Aspergillus nidulans* as well and the DNAs in these cases were also readily digested with the restriction enzymes (data not shown).

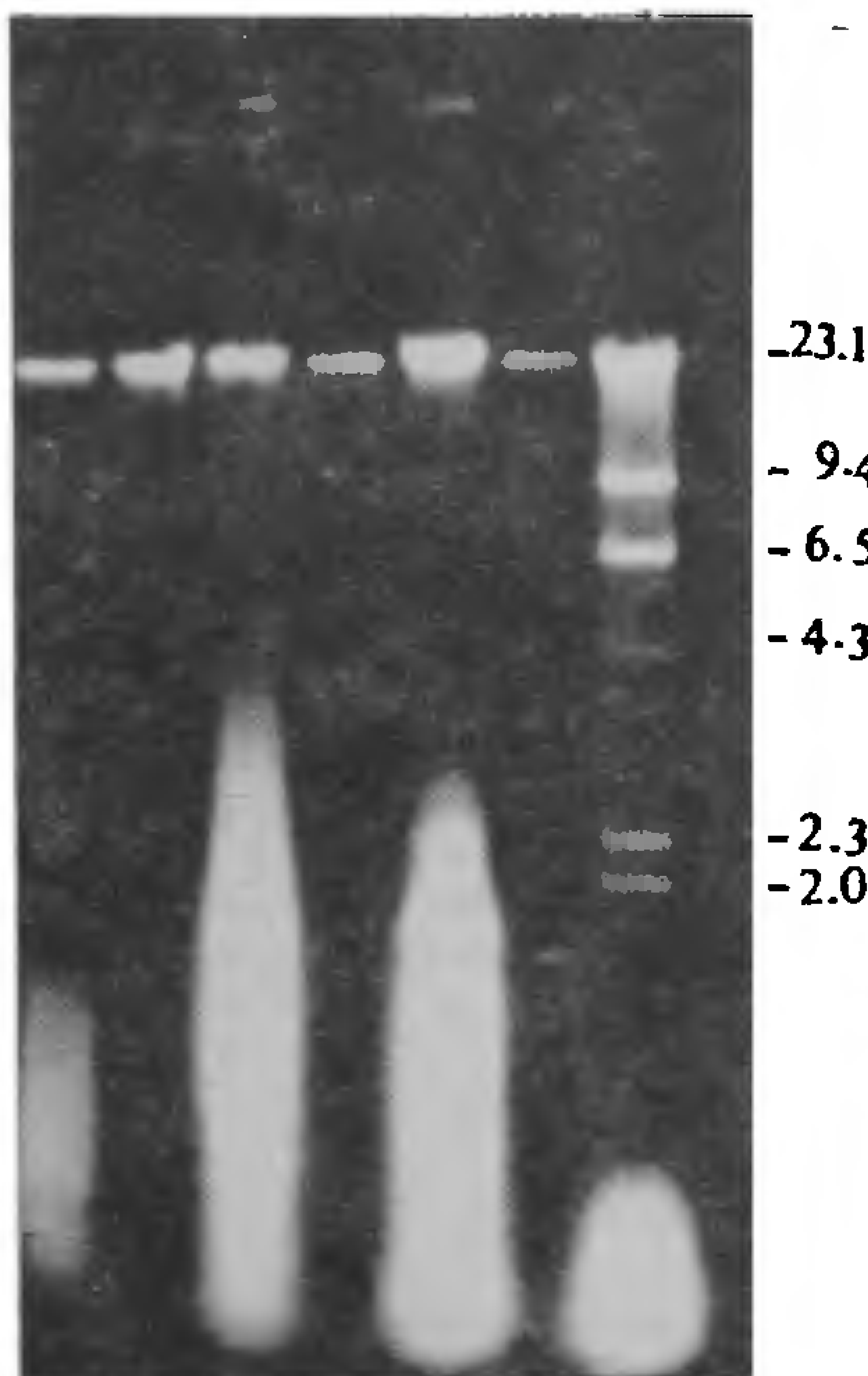

Figure 1. Isolation of fungal DNA by CTAB and TENS methods. The DNA was isolated from *T. lanuginosus* as described in the text. ***a***, ***b***, by CTAB procedure (2.3 μg and 0.7 μg, respectively); ***c***, ***d***, by TENS procedure (9.6 μg and 1.15 μg, respectively); ***e***, ***f***, by hot TENS procedure (16.8 μg and 0.8 μg, respectively); ***b***, ***d***, ***f***, after RNase digestion. M – Lambda DNA (crude preparation) digested with HindIII.

Thermomyces lanuginosus was maintained on sucrose–asparagine medium[7]. Mycelial inoculum was prepared by incubating the spores in Vogel's medium[8] containing 0.2% yeast extract on a gyratory shaker at 200 rpm for 15 h at 50°C. The fungus was grown in 100 ml Vogel's medium in a 500 ml Erlenmeyer flask for 15 h at 50°C using 3% mycelial inoculum. *Aspergillus nidulans* was maintained on 2% Vogel's starch agar[8] and grown in 2% Vogel's -sucrose medium at 32°C for about 15 h with shaking. *Saccharomyces cerevisiae* was maintained on YEPD agar and grown in YEPD medium for about 12 h with shaking and harvested as described by Rothstein[1]. The mycelial cultures

Table 1. The yield and purity of DNA isolated from *T. lanuginosus* using different procedures

Method	Absorption ratio (260/280)	Yield (mg/g wet wt.)
CTAB	1.72	0.913
TENS	1.65	14.041
Hot TENS	1.60	6.225
After RNase treatment		
CTAB	1.87	0.134
TENS	1 35	2 288
Hot TENS	1.78	2 801

Figure 2. Restriction enzyme digestions of the DNA. The DNA obtained after RNase treatment was used for restriction enzyme digestion. ***a***, ***d***, ***g***, DNA of CTAB procedure; ***b***, ***e***, ***h***, DNA of TENS procedure; ***c***, ***f***, ***i***, DNA of hot TENS procedure; ***d***, ***e***, ***f***, after digestion with the restriction enzyme Pst1 for 3 h at 37°C; ***g***, ***h***, ***i***, after digestion with the restriction enzyme Sau3A1 for 15 min at 37°C

were harvested by suction filtration on Whatman No 1 filter paper, washed twice with distilled water, pressed between a few layers of blotting paper, weighed and stored at –70°C.

In the CTAB (cetyltrimethyl ammonium bromide) procedure nucleic acids were extracted essentially as described by Rogers and Benedich[6] with the following modifications: PVP was omitted from the buffer and all centrifugations were done at room temperature (30 ± 3°C) except for the last centrifugation, which was done at 4°C.

The TENS procedure is as follows:

1. Grind the frozen mycelia with twice the weight of sand into a fine powder using a pre-chilled pestle and mortar and transfer the powder into a 50 ml polypropylene tube.
2. Add 10 volumes of TENS (0.05 M Tris (pH 8.0), 0.1 M EDTA (pH 8.0), 0.1 M NaCl and 1% SDS) buffer and mix thoroughly and gently.
3. Add an equal volume of neutral phenol, mix gently and place the tube at –20°C for 30 min.
4. Thaw the contents of the tube and centrifuge for 10 min at 12,000 *g* at room temperature (30 ± 3°C).
5. Extract the upper layer with phenol: chloroform: isoamyl alcohol (25 24 1).
6. Centrifuge for 10 min at 12,000 *g* at room temperature (30 ± 3°C)
7. Transfer the upper layer to a fresh tube and add 1/10 volume of 3 M potassium acetate (pH 4.8).
8. Precipitate the nucleic acids with 2 volumes of distilled, cold ethanol.
9. Centrifuge for 15 min.
10. Dry the pellet and dissolve in 0.1 × TE (10 mM Tris (pH 8 0) and 1 mM EDTA (pH 8.0)).

For hot TENS procedure, TENS buffer at 65°C was added and incubated at 65°C for 10 min and then extracted with an equal volume of neutral phenol. The upper layer was processed as above from steps 5–10.

A modified procedure for DNA isolation from higher fungi, using hexadecyltrimethyl ammonium bromide has been reported after submission of this note[9].

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The recent rise of the river bed near Mohand, Western Uttar Pradesh

Himalayan foreland basin between the rivers Ganga and Yamuna in the western Uttar Pradesh has many strike-slip faults cutting across the Siwalik range. One such fault, a small en echelon fault associated with Dhaulkhand fault zone, runs along Gajrao in its upper catchment area. A definite right lateral slip along this fault brings a northeasterly dipping clay band opposite to the sandstone bed. A major portion of the dislocated sandstone hillock rests precariously on the dip-slope provided by the underlying moistened clay band. The impounding enormous pressure on the substratum squeezes and uplifts these clays and the overlying recent sediments in the river bed abutting against the fault

It was January the 19th, 1994, when most of the news dailies in Dehradun valley carried a sensational news on the front page that a forest land near Mohand in Siwalik foothills is rising up abnormally and has gained a height of about 8 m above the stream level. This news caused panic amongst the populace sprawling in the nearby region (based on the FIR lodged at Mohand Police Station and Forest Check Post by Mr Mir Ali) as they suspected a catastrophy in the near future in the form of a volcano or related activity.

Mr Mir Ali, a Gujjar, who hails from the area of the present activity, has been an eyewitness to this whole event which, according to him, first started in 1992 monsoon time (July–August). During that period, it attained a height of about 1.5 m before being washed out. Later, during 1993 monsoon time, the process was again reactivated at the same place and till October 1993 it kept on rising to a considerable height. The rise also produced noise, he said. Thereafter, he did not notice any significant change in height; however, fractures started appearing in the elevated rock mass. In 1990, an event of such unusual rise of the river bed was also reported from Tal valley[1], SE of Dehradun in Garhwal Lesser Himalaya.

To have a first-hand account of the operating processes in this region, the present team carried out geological investigations. These studies are presented in this paper.