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Potential application of β-1, 3 glucanase from an environmental isolate of *Pseudomonas aeruginosa* MCCB 123 in fungal DNA extraction

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Pseudomonas aeruginosa MCCB 123 was grown in a synthetic medium for β-1,3 glucanase production. From the culture filtrate, β-1,3 glucanase was purified with a molecular mass of 45 kDa. The enzyme was a metallozyme as its β-1,3 glucanase activity got inhibited by the metal chelator EDTA. Optimum pH and temperature for β-1,3 glucanase activity on laminarin was found to be 7 and 50 °C respectively. The MCCB 123 β-1,3 glucanase was found to have good lytic action on a wide range of fungal isolates, and hence its application in fungal DNA extraction was evaluated. β-1,3 glucanase purified from the culture supernatant of *P. aeruginosa* MCCB 123 could be used for the extraction of fungal DNA without the addition of any other reagents generally used. Optimum *p*H and temperature of enzyme for fungal DNA extraction was found to be 7 and 65 °C respectively. This is the first report on β-1,3 glucanase employed in fungal DNA extraction.

Keywords: β-1,3 glucanase, DNA extraction, Fungus, *Pseudomonas aeruginosa*

β-1,3 glucanases represent a well-known class of enzymes widespread in bacteria and fungi, and they are hydrolases specific to O-glycoside bonds between 1,3-linked glucopyranose residues found in a variety of β-glucans[1]. Exo-β-1,3 glucanases cleave glucose residues from non-reducing ends degrading the polysaccharides completely and releasing monosaccharide residues, while, endo-β-1,3 glucanases cleave β-linkages at random sites along polysaccharide chain releasing short oligosaccharides[2,3].

Bacterial and fungal β-1,3 glucanases are involved in the degradation of polysaccharides present in their natural environment and used as an energy source[4]. Several strains of bacteria are able to lyse and grow on viable yeast and fungal cells by producing a variety of cell wall degrading enzymes such as endo-β-1,3 glucanases, β-1,6 glucanases, mannanases and chitinases[5]. A glucanase producing strain of *Pseudomonas aeruginosa* was used in the biological control of cyst forming nematode *Heterodera cajani* on sesame[6]. β-1,3 glucanase produced by *Pseudomonas aeruginosa* PN1 is reported to cause mycelial lysis, vacuolation and granulation of cytoplasm, hyphal deformities and branching in polyphagous fungus *Macrophomina phaseolina*[7]. Mycolytic enzymes produced by antagonistic microorganisms are very important in bio-control technology[8].

Jose[9] found *P.aeruginosa* MCCB 123 to be a potential producer of β-1,3 glucanase having lytic action on the cell wall of a wide range of fungi and therefore an evaluation has been made in the application of this enzyme in fungal DNA extraction.

Materials and Methods

Enzyme production—Absorbance of an 18 h old broth culture of *Pseudomonas aeruginosa* MCCB 123 was adjusted to 0.1 at Abs_{600}. The flasks were inoculated with 1% v/v (final) of the culture to get cell count of 1×10^7CFU/mL. β-1,3 glucanase was produced in a synthetic medium composed of (gL^{-1} distilled water) glucose,7.5; yeast extract, 2.5; $NH_4H_2PO_4$, 10.04; Na_2HPO_4, 0.5; KH_2PO_4, 3.0; $MgSO4.7H_2O$, 0.2; $CaCl_2$, 0.000625; $ZnCl_2$, 0.01; casein, 10.0; *p*H, 7.0 in a 5-L fermenter (Biostat-B-Lite, Sartorius, Germany). Fermentation was carried out at 25 °C, *p*H 7.0±0.05, 300 rpm supplied with sterile air at the rate 2.5 L min^{-1}. For enzyme extraction the culture was centrifuged at 8260 *g* for 15 min at 4 °C and the supernatant stored in 300 mL aliquots at -20 °C, and used for further purification and characterization.

Purification of β-1, 3 glucanase—Partial purification of the enzyme was carried out by

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precipitation of the cell-free culture supernatant with ammonium sulphate between 30-80% saturation. The precipitates were collected by centrifugation at 8260 *g* for 15 min at 4 °C and the active fractions were pooled and resuspended in 20 m*M* Tris-Cl buffer, at *p*H 8.5. The partially purified enzyme was dialyzed against 20 m*M* Tris-Cl buffer, at *p*H 8.5 using Amicon UF stirred cell (Millipore Corporation, USA, Model 8010), with a 10 kDa cut off membrane (Omega, 25 MM, 10K, Pall life sciences) and used for further purification. The enzyme was then loaded on an AKTA Prime protein purification system equipped with a C16/40 (16 mm×40 cm) (GE Healthcare Biosciences, Uppsala) DEAE cellulose (Sigma–Aldrich Co.) column equilibrated with 20 m*M* Tris-Cl buffer, at *p*H 8.5. The column was washed with the same buffer to remove the unbound proteins, and the enzyme was eluted by applying a linear gradient of NaCl from 0–1000 m*M* at a flow rate of 0.5 mL/min, and fractions of 2 mL were collected. β-1,3 glucanases fractions were pooled and concentrated by lyophilization.

β-1,3-glucanase assay—β-1,3 glucanase activity was measured by using laminarin from *Laminaria digita* (Sigma–Aldrich Co.) as substrate according to the modified protocol of Zhu *et al*[1]. The reaction mixture consisting of 1mg mL^{-1} laminarin and 0.5 mL of 5 mg mL^{-1} β-1,3 glucanase (dissolved in 50 m*M* sodium-phosphate buffer, *p*H 6.0) and incubated at 50 °C for 30 min. After incubation, 1 mL DNS reagent was added and tubes were placed in boiling water bath for 10 min, cooled and 4 mL of distilled water was added and the amount of reducing sugar liberated was measured at 540 nm. Assays were carried out in triplicates. One unit of enzyme activity is defined as the amount of enzyme that catalyzed the liberation of reducing sugar equivalent to 1 µg D-glucose per minute under standard assay conditions.

Characterization of β -1, 3 glucanase

Determination of molecular weight—The lyophilized active fractions of the enzyme were subjected to reducing sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) as per Laemmli[10].

Protein assay—Quantification of protein was carried out according to the method of Hatree[11] using bovine serum albumin as standard.

Specific activity—Specific activity was calculated by dividing the enzyme units with the protein content.

Effect of pH on β-1,3 glucanase activity—Effect of *p*H on β-1,3 glucanase activity was determined over a *p*H range of 3-10 using the buffers of 50 m*M* concentrations: sodium–phosphate (6,7), Tris-Cl (8 and 9), glycine-NaOH (9, 10, 11 and 12) for 30 min at 37 °C.

Effect of temperature on β-1,3 glucanase activity—Effect of temperature on β-1,3 glucanase activity was tested by carrying out the assay at temperature ranges of 30, 40, 50, 60, 70 and 80 °C for 30 min in 50 m*M* Tris-Cl buffer (*p*H 9.0).

Effect of inhibitors on β-1,3 glucanase activity—Inhibitory action of 5 m*M* phenyl methyl sulphonyl fluoride (PMSF), EDTA, 1,10 phenanthroline, leupeptin, pepstatin, phosphoramidon and TLCK was investigated by including them in the β-1,3 glucanase assay mixture, and the relative activity measured under standard assay conditions. Untreated enzyme was taken as the control (100% activity).

Cytotoxicity analysis of purified β-1,3-glucanase—HeLa cells were seeded in 96 well plates (Greiner Bio-One) containing 82 m*M* glutamine, 1.5 g L^{-1} sodium bicarbonate and 10% fetal bovine serum. Purified enzyme in concentrations of 0-250 µg mL^{-1} (v/v) was added to the wells in triplicates. A control was kept without the enzyme addition. After 14 h incubation MTT assay was performed and the percentage of inhibited cells at each concentration of the protease was calculated using SPSS software (SPSS package for Windows).

MTT assay—After replacing the medium, 50 µL MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (Sigma-Aldrich Co.) having a strength of 5 mg mL^{-1} in PBS was added to each well and incubated for 5 h in dark. MTT was added to the control wells with the medium alone. The medium was removed and MTT-formazan crystals were dissolved in 200 µL dimethylsulfoxide. Absorbance was recorded immediately at 570 nm in micro plate reader (TECAN Infinite Tm, Austria). Probit analysis for percentage cell inhibition was done with SPSS software package (version 17).

Application of MCCB 123 β-1, 3 glucanase in fungal DNA extraction

Standardization of pH, temperature and incubation time for cell lysis by β-1,3 glucanase on fungus Saccharomyces cervisiae MTCC 1766 as the reference strain—Lytic activity was carried out according to the modified method of Niwa *et al*[12]. *Saccharomyces cervisiae* MTCC 1722 was grown for

48 h at 28 °C. The absorbance of cell suspension was adjusted to 1.0 at Abs_{600}, centrifuged at 15,000 *g* at 4 °C for 15 min and the pellets recovered. For *p*H optimization, cells were suspended in 1 mL of β-1,3 glucanase (10 mg enzyme suspended in 50 m*M* sodium acetate of *p*H 5-6, 50 m*M* Tris-Cl of *p*H 7-10) and incubated for 30 min at 25 °C. For temperature optimization, cells were suspended in 1 mL purified β-1,3 glucanase (10 mg enzyme suspended in 1 mL 50 m*M* Tris-Cl, *p*H 7.0) and incubated for 30 min at various temperatures ranging from 25-75 °C. To determine optimum incubation time for cell lysis, the cells were suspended in 1 mL of β-1,3 glucanase (10 mg enzyme suspended 1 mL 50 m*M* Tris-Cl, *p*H 7.0) at 35 °C and incubated up to 60 min, drawing samples for DNA extraction at every 10 min interval.

After each experiment, un-lysed cells were removed by centrifugation at 15,000 *g* for 15 min at 4 °C. Into the supernatant equal volume of absolute ethanol was added, kept for 30 min and the pellet was recovered by centrifugation at 15,000 *g* for 15 min at 4 °C and dissolved in 100 µL sterile Milli Q, and the presence of DNA was confirmed on 1% agarose gel and the DNA yield was determined by triplicate measurements at 260 nm. Reactions without enzyme were included as controls. Optimum was determined based on band intensity and DNA yield. The band intensity was calculated using Quantity one software, BioRad, USA.

DNA extraction from fungal cultures—Fungal cultures used for DNA extraction and their culture conditions are listed in Table 3. Fungal cultures were grown until enough fungal mycelia could be generated. Aliquots of 1mL each of the cultures were centrifuged at 15,000 *g* at 4 °C for 15 min and mycelia having uniform quantity were treated with 1 mL (10 mg enzyme mL^{-1}) column purified glucanase resuspended in 50 m*M* Tris-Cl having *p*H 7.0 and incubated at 65 °C for 60 min. DNA extraction and yield determination were carried out by the method as described earlier.

Nucleic acid yield and purity—Nucleic acid extracted from fungal isolates was quantified using UV-visible spectrophotometer (UV-1601, Shaimadzu). The absorbance at 260 nm (Abs_{260}) was measured for each sample and used to calculate the average total nucleic acid yield for each set of triplicate samples. To estimate the purity of extracted nucleic acid, the absorbance at 280 nm (Abs_{280}) was measured and the average ratio between the Abs_{260} nm and Abs_{280} nm (Abs_{260}/Abs_{280}) was calculated for each set of triplicate samples.

PCR amplification of ITS region—PCR amplification of ITS region of the extracted DNA from fungi consisting of ITS 1 and ITS 2 was performed according to White *et al.*[13] using primers ITS1 (5' TCC GTA GGT GAA CCT GCGG-3') and ITS4 (5' TCC TCC GCT TAT TGA TAT GC-3'). The amplification profile consisted of initial denaturation at 95 °C for 5 min followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 56 °C for 45 sec and extension at 72 °C for 1 min followed by final extension at 72 °C for 10 min, and the PCR products were separated on 1% agarose gel.

Microscopic examination of β-1,3 glucanase treated fungal hyphae and yeast cells—For the examination of cell rupture, lysed cells were observed under phase contrast microscope (Olympus) and compared with those of the controls (untreated cells).

Statistical analysis—Data were analyzed using one-way Analysis of Variance (ANOVA) with post-hoc multiple comparison analysis performed using Tukey's HSD. Mean of the results was compared using SPSS 17.0 package for Windows at a significance level of $P<0.05$. Data are presented as mean± SD.

Results

Purification of β-1,3 glucanase—The enzyme was purified by a two step procedure, initially by precipitation with ammonium sulphate and subsequently by DEAE-cellulose column chromatography. Ammonium sulphate fractions from 30-80% showed β-1,3 glucanase activity. Active fractions were pooled and concentrated by ultrafiltration using a 10 kDa membrane. The pooled fractions were then loaded into DEAE-cellulose chromatography column. The results of purification procedure are summarized in Table 1. The enzyme was purified with 9.52 fold increase in specific activity. The elution profile of the β-1,3

Table 1—Purification profile of β-1,3 glucanase of *Pseudomonas aeruginosa* MCCB 123

Purification step	β-1,3 glucanase activity(U mL^{-1})	Total protein (mg)	Specific activity (Umg^{-1})	Purification fold
Culture filtrate	359.71	8.5	42.31	0
$(NH4)_2SO_4$ Precipitation	188.08	4.09	45.98	1.08
DEAE-cellulose chromatography	98.74	0.245	403.02	9.52

glucanase on DEAE-cellulose column is shown in Fig. 1. The enzyme eluted between 0.70 *M* - 0.81 *M* NaCl (fractions, 70 to 81) contained β-1,3 glucanase.

Characterization of β-1,3 glucanase—The purified MCCB 123 β-1,3 glucanase was homogenous on SDS-PAGE and its molecular weight was estimated to be 45 kDa by reducing SDS-PAGE (Fig. 2).

Effect of pH on activity of β-1,3 glucanase—The effect of *p*H on β-1,3 glucanase activity was determined using buffers in the *p*H range of 6-12 at 50 °C. The enzyme was found to exhibit activity from

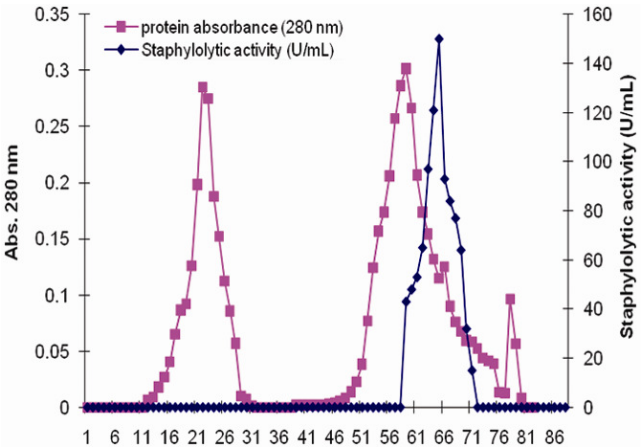

Fig.1—Elution profile of β-1,3 glucanase on DEAE-cellulose C16/40 column.

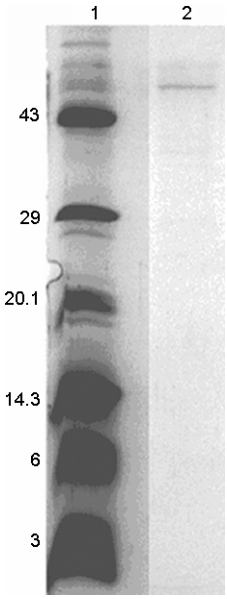

Fig. 2—SDS-PAGE profile of purified LasA protease. Lane 1, Molecular weight marker; lane 2, 45 kDa β-1,3 glucanase enzyme.

*p*H 3-10 with its optimum at *p*H 7.0. Statistical analysis by One-way ANOVA indicated that there was a significant (P<0.05) difference in the β-1,3 glucanase activity between *p*H 3-7.

Effect of temperature on activity of β-1,3 glucanase—The enzyme was found to exhibit activity from 30 to 80 °C with its optimum at 50 °C. There was a significant (P<0.05) difference in the β-1,3 glucanase activity in temperature ranges between 30–50 °C.

Effect of inhibitors on activity of β-1, 3 glucanase—There was a partial inhibition (42.98%) of enzyme activity by metalloprotease inhibitor EDTA thus proving to be metalloprotease. The enzyme retained 83.34, 84.22, 81.51 and 95.62 % activity in presence of 5 m*M* 1, 10 phenanthroline, 50 µ*M* leupeptin, 10 µ*M* pepstatin and 0.1 m*M* phosphoramidon, respectively, confirming that the enzyme did not belong to the class of serine and cysteine protease, respectively (Table 2).

Cytotoxicity analysis of purified β-1, 3-glucanase—Cytotoxicity analysis revealed that 236.87±1.89 µg mL^{-1} was the LD_{50} dose (50 % inhibition).

Application of β-1, 3 glucanase in fungal DNA extraction—Optimization of *p*H for DNA extraction was accomplished over a *p*H range of 5-10 using 50 m*M* sodium acetate for *p*H 5-6, 50 m*M* Tris-Cl for *p*H 7-10 at 25 °C for 30 min. The enzyme exhibited good lytic activity on cells of *Saccharomyces cervisiae* from *p*H 5-10 with its optimum at 7.0 with a DNA yield of 231.66±5.20 µg mL^{-1}. The statistical analysis revealed that *p*H imposed a significant (P<0.05) difference in the DNA yield between *p*H 8-9. However, there was no significant difference in the DNA yield between *p*H 5-10. The enzyme was found to have good cell lysis from 25-75 °C with its optimum at 65 °C with a DNA yield of 310±2.5 µg mL^{-1}. There was a significant (P<0.05)

Table 2—Effect of inhibitors on β-1,3 glucanase activity

Inhibitors	Concentration	Relative activity (%)
Control		100
EDTA	10 m*M*	57.02
PMSF	2 m*M*	100
1, 10 phenanthroline	5 m*M*	83.34
TLCK	0.1 m*M*	100
Leupeptin	50 µ*M*	84.22
Pepstatin	10 µ*M*	81.51
phosphoramidon	0.1 m*M*	95.62

difference in the DNA yield at a temperature range of 45–65 °C. The DNA yield reached maximum after 60 min incubation (321.66±5.2 µg mL^{1}). The DNA yield was significantly (P<0.05) different between a range of 20-30 min incubation.

The gel image of DNA extracted at various *p*H (Fig. 3a), temperature (Fig. 3b) and incubation time (Fig. 3c) is represented.

DNA extraction from different fungal species—The DNA extracted from various species of fungi (Fig. 4) along with their DNA yield and qualities are described in Table 3.

Microscopic examination of β-1,3 glucanase treated ruptured hyphae and yeast cells—For the examination of fungal cell rupture on treatment with β-1,3 glucanase, lysed cells were observed under phase contrast microscope and compared with that of control (untreated cells). The effect of β-1,3 glucanase enzyme on fungal cells is represented in Fig. 5. The cells lost their normal appearance on treatment with the enzyme compared to that of the control.

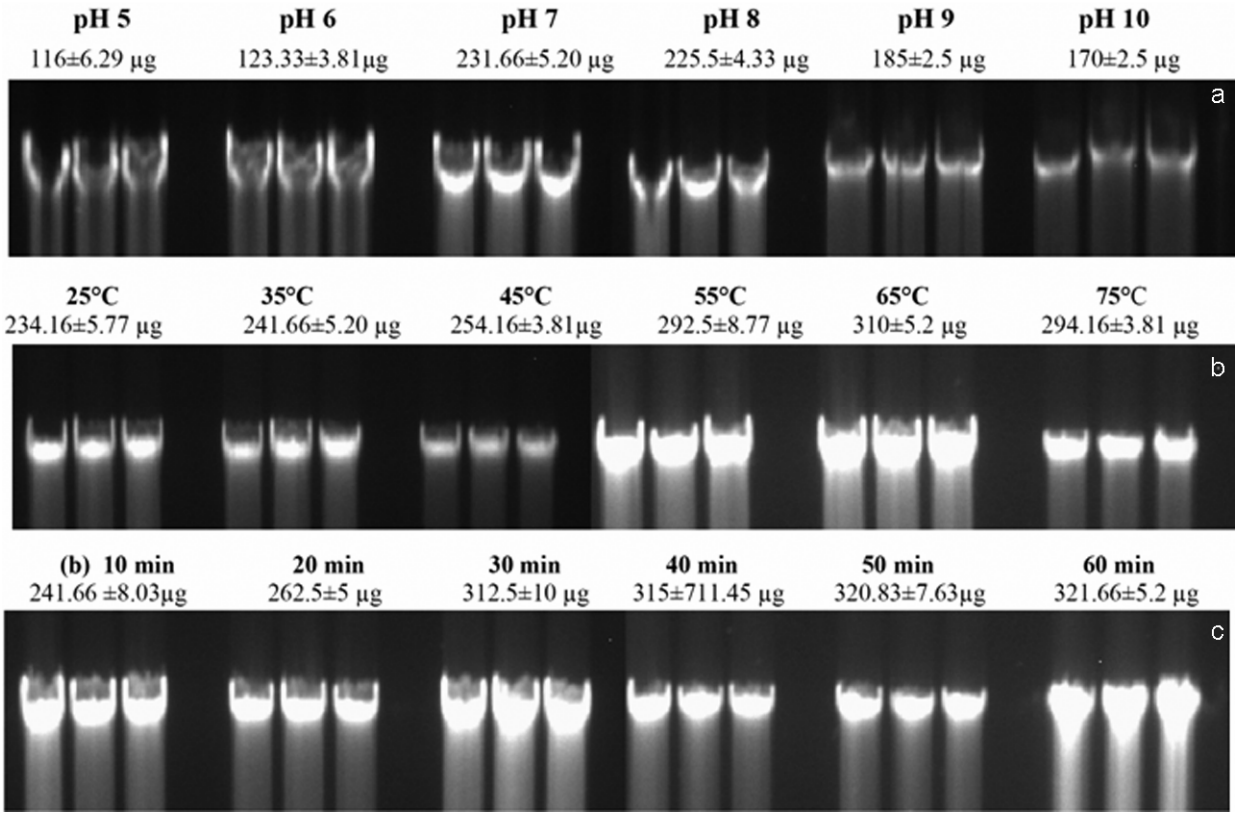

Fig. 3—Optimum *p*H (a), temperature (b) and incubation time (c) for cell lysis for fungal DNA extraction using *Sacchromyces cervisiae* MTCC 1766 as reference strain. Concentration of DNA in µg $µl^{-1}$ (average ±SD) is represented.

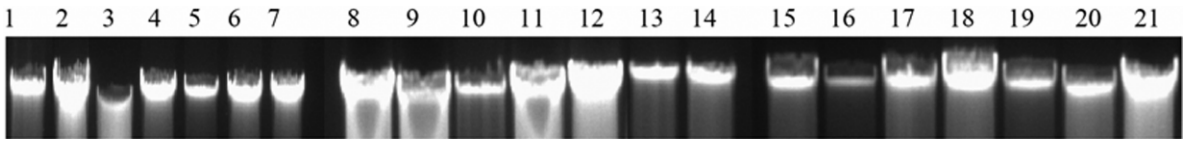

Fig. 4—DNA extracted from fungal isolates by the lytic action of β-1,3 glucanase. Lane 1, *Aspergillus flavus* MTCC 277; lane 2, *Aspergillus foetidus* MTCC 151; lane 3, *Fusarium solani* MTCC 350; lane 4, *Acremonium diospyri* MTCC 1316; lane 5, *Phanerochaete chrysosporium* MTCC 787; lane 6, *Candida albicans* MTCC 854; lane 7, *Saccharomyces cerevisiae* MTCC 1766; lane 8, *Pleurotus sajor-caju* MTCC 141; lane 9, *Pleurotus sajor-caju* MTCC 1806; lane 10, *Pleurotus sapidus* MTCC 1807; lane 11, *Pleurotus fossulatus* MTCC 1800; lane 12, *Pleurotus ostreatus* MTCC 1803; lane 13, *Trametes hirsute* MTCC 136; lane 14, *Coriolus versicolor* MTCC 138; lane 15, *Aspergillus ochracens* MTCC 1810; lane 16, *Pencillium citrinum* MTCC 2553; lane 17, *Trichoderma reesei* MTCC 164; lane 18, *Heterobasidion annosum* MTCC 146; lane 19, *Pycnoporus sanguineus* MTCC 137; lane 20, *Pleurotus osterus* MTCC 142; lane 21, *Daedalea flavida* MTCC 145.

Table 3 — Fungal species used for DNA extraction. DNA yield and quality

Fungal species	Code	Culture Conditions	Temp (°C)	DNA yield ($\mu g\ \mu L^{-1}$)	DNA purity ($Abs_{260}/_{280}$)
Saccharomyce cerevisiae	MTCC 1766	YEPD	30	280±7.5	1.10±0.03
Candida albicans	MTCC 854	MYA	37	266.66±5.20	1.14±0.03
Aspergillus flavus	MTCC 277	CYA	30	164.16±8.77	1.07±0.04
Aspergillus foetidus	MTCC 151	CYA	30	188.33±8.77	1.10±0.07
Aspergillus ochracens	MTCC 1810	CYA	35	154.16±3.81	1.16±0.02
Phanerochaete chrysogenum	MTCC 787	MEA	25	156.66±7.63	1.11±0.07
Fusarium solani	MTCC 350	PSA	30	170.83±10.10	1.13±0.05
Acremonium diospyri	MTCC 1316	PDA	25	311.66±8.77	1.19±0.003
Heterobasidion annosum	MTCC 146	YGA	25	183.33±5.20	1.12±0.01
Trichoderma reesei	MTCC 164	MEA	25	134.16±3.81	1.10±0.02
Pencillium citrinum	MTCC 2553	CYA	30	146.66±3.81	1.17±0.05
Pleurotus sajor-caju	MTCC 141	PDA	25	218.33±5.20	1.17±0.04
Pleurotus sajor-caju	MTCC 1806	PDA	25	227.5±5	1.08±0.02
Daedalea flavida	MTCC 145	YGA	25	247.5±5	1.07±0.01
Pleurotus ostreatus	MTCC 142	YGA	30	226.66±3.81	1.11±0.03
Pleurotus sapidus	MTCC 1807	PDA	25	224.16±3.81	1.11±0.02
Pleurotus ostreatus	MTCC 1803	PDA	25	255.83±5.77	1.23±0.02
Pleurotus fossulatus	MTCC 1800	PDA	25	226.66±5.20	1.10±0.006
Coriolus versicolor	MTCC 138	YGA	25	219.16±5.20	1.10±0.02
Trametes hirsuta	MTCC 136	YGA	25	240.83±6.29	1.06±0.02
Pycnoporus sanguineus	MTCC 137	YGA	25	219.16±6.29	1.11±0.05

CYA: Czapek Yeast Extract Agar, PSA: Potato Sucrose agar, MYA: Malt Yeast agar
PDA: Potato Dextrose agar, YGA : Yeast glucose agar

Discussion

A β-1, 3 glucanase with a broad range of lytic activity on various species of fungal cell walls was purified from ***P. aeruginosa*** MCCB 123. The enzyme was purified with 9.52 fold increase in specific activity. The molecular mass of the enzyme was found to be 45 kDa by SDS-PAGE. The enzyme was proved to be a metallozyme, since the β-1,3 glucanase activity was inhibited by the zinc chelator, EDTA. The optimum *p*H for β-1,3 glucanase activity on the substrate laminarin was 7.0 and temperature 50 °C. These *p*H and temperature optima are different from the ones (acidic *p*H and 40 °C) reported for ***Pseudomonas stutzeri*** YPL-1[14].

β-1,3 glucanase of ***P. aeruginosa*** MCCB 123 was found to have lytic action on a broad range of fungi and yeasts. β-1,3 glucanase alone is not sufficient for lysis and it need the assistance of protease[15]. However, β-1,3 glucanase of ***P. aeruginosa*** MCCB 123 is alone sufficient for the lysis of yeast cell as evidenced from its lytic action on the yeasts such as ***Saccharomyces cerevisiae*** and ***Candida albicans***. Type 1 β-1,3 glucanases are capable of readily solubilising yeast glucan and inducing complete lysis of viable yeast cells, while members of type II β-1,3 glucanases have limited capacity to solubilise glucan[16]. Thus, MCCB 123 β-1,3 glucanase belongs to type I β-1,3 glucanase since it exhibited good hydrolytic activity on yeasts, ***Saccharomyces cerevisiae*** and ***Candida albicans***.

Due to the highly complex nature of fungal cell wall, most enzymes fail to lyse, and hence, development of a single universal fungal DNA extraction method has not been accomplished so far. This has paved the way for developing DNA extraction methods using a combination of different disruption techniques[17-19]. Most of the lysis buffers for DNA extraction include SDS[20-22]. Moreover, the existing methods for genomic DNA preparation from fungi require several hours for completion[23,24].

Therefore, in this context developing a single extraction method for fungal DNA is a desirable proposition. DNA

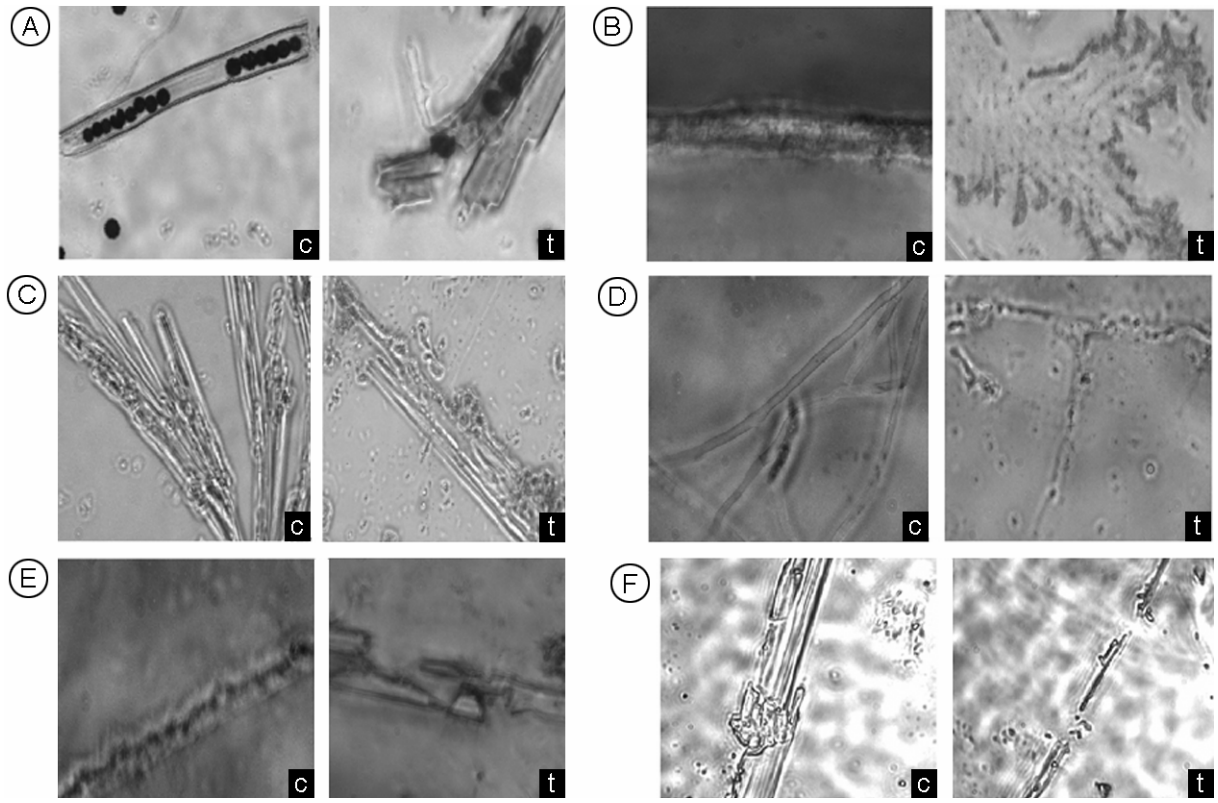

Fig. 5—Rupture of fungal hyphe on treatment with β-1,3 glucanase of *Pseudomonas aeruginosa* MCCB 123. Control (c) represents untreated fungal hyphae and test (t) represents the changes in fungal hyphae on treatment with 10 mg mL^{-1} of purified β-1,3 glucanase [A= *Aspergillus foetidus* MTCC 151, B= *Aspergillus flavus* MTCC 277, C= *Acremonium dyosprii* MTCC 1316 , D= *Fusarium solani* MTCC 350, E= *Phanerocheate chrysogenum* MTCC 787, F= *Pleurotus fossulatus* MTCC 1800] Magnification 600X.

could be extracted from 21 fungal species by the lytic action of the purified β-1,3 glucanase from *P. aeruginosa* MCCB 123 without the addition of any other reagent and implementation of mechanical treatments as well, transforming this method unique among the ones reported. The extracted DNA could be directly used for PCR amplification without further purification. Moreover, the method is less expensive as it employs only β-1,3 glucanase as the sole reagent, the quality and quantity of DNA obtained is suitable for molecular assays and it doesn't require the use of specialised equipments or hazardous reagents.

The quality of the extracted nucleic acid is important for further processing. Samples with mean A_{260}/A_{280} ratios below 1.8 were presumed to contain protein or other contaminants, whereas samples with ratios above 2.0 were presumed to be due to the presence of RNA. However, nucleic acids preparations free of phenol should have Abs $_{260}/_{280}$ ratios near 1.2[24,25]. In the case of DNA extracted with β-1,3 glucanase from various fungal species, $Abs_{260}/_{280}$ ratios were with in the range of 1.0 to 1.1. As phenol had not been used in the process it could be concluded that the DNA extracted using this method was free of contamination and was suitable for PCR.

Fungal DNA extraction using MCCB 123 β-1,3 glucanase has several advantages. The number of steps in DNA extraction procedure was minimized by replacing phenol chloroform extraction method and it also did not involve the addition of any detergent, lytic agents and implementation of other mechanical lytic methods such as grinding with sand, repeated freeze thaw cycles in liquid nitrogen etc. Several samples could be processed within a short time period of 30 min. The method yielded high quality DNA suitable for PCR. This method is likely to be cost-effective since β-1,3 glucanase alone needs to be used as the sole reagent to a broad range of fungi. These properties qualify the enzyme unique over all other lytic enzymes used for DNA extraction from fungi. This is the first report of a lytic enzyme being employed solely in fungal DNA extraction without any additives. The broad range of lytic activity of β-1,3 glucanase on a wide range of fungi has immense benefits in DNA extraction in commercial point of view.

Conclusion

Development of a single universal fungal DNA extraction method has significance and has not been accomplished so far. There is no single protocol appropriate for cell lysis for all fungi and each species requires a specific method for efficient DNA extraction. β-1,3 glucanase from *P. aeruginosa* MCCB 123 was found to have lytic action on a broad range of fungal and yeast strains. This could be exploited in DNA extraction from fungi without the addition of other reagents, and by incorporating other mechanical lytic steps. Therefore, broad range of lytic action of MCCB 123 β-1,3 glucanase has immense benefits in fungal DNA extraction for the development of a single universal protocol.

Acknowledgement

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