Short communication

High-Molecular-Weight Genomic DNA Isolation from *Doratomyces microsporus* and Synthesis of a Genomic DNA Library

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Abstract

A protocol for isolation of genomic DNA from a filamentous fungus was optimised and a genomic library was constructed. Various methods for preparing genomic DNA from different fungi are known: simplified methods to allow processing of large sample numbers and methods to increase the quality of DNA, which is especially important for synthesis of a genomic DNA library. An isolation of high-molecular-weight DNA from a fungus is a challenging process since cell wall composition and cellular components differ with the fungal species. A procedure for isolation of high-molecular-weight DNA from the keratinolytic filamentous fungus *Doratomyces microsporus*, strain MZKI B399, is described and compared to the method used for filamentous bacteria and to the method including commercially available DNA extraction buffer. The isolation protocol that gave the desired quality of DNA was optimised in the following steps: disruption of cells by grinding in liquid nitrogen, followed by removal of polysaccharides and proteins by a phenol extraction buffer and finally precipitation of DNA with isopropanol. The obtained DNA was used to produce the bacteriophage genomic DNA library of the fungus.

Keywords: Keratinolytic fungus, *Doratomyces microsporus*, genomic DNA library, DNA isolation

1. Introduction

Molecular biological studies of organisms with an as yet unsequenced genome require construction of a genomic DNA library. Isolation of high-molecular-weight genomic DNA from filamentous fungi is problematic due to a rigid cell wall, high polysaccharide content[1] and presence of nucleic acid hydrolysing enzymes. Numerous procedures for fungal DNA isolation have been developed that more or less successfully overcome the described difficulties. Due to specific characteristics of each particular group of fungi, a specific method often has to be designed for the chosen group.[2–7]

For disruption of fungal cell wall, an enzymatic[2–5] or a mechanical force, such as sequential rapid freezing and thawing (for yeasts),[8,9] grinding lyophilized mycelium,[6] and disruption of cell walls in micro-dismembrators[7] could be used. The cell wall- degrading enzymes are used to form spheroplasts that can easily be disrupted with extraction buffers. However, a more efficient but time-consuming method for cell wall disruption is grinding the frozen mycelium.

Cetyltrimethylammonium bromide (CTAB) is usually used for removal of polysaccharides.[10,11] Proteinase K[6,12,13] or phenol along with detergents,[7] or benzyl chloride[14] are used for removal of proteins. However, high-molecular-weight DNA is essential for construction of a genomic DNA library therefore a high number of steps in the procedure should be avoided due to higher risk of shearing the DNA molecules.

A deuteromycetous fungus *Doratomyces microsporus* produces a serine proteinase with a high keratinolytic activity[15–17] known to hydrolyse keratinous and also some non-keratinous proteins. Keratinases are important enzymes with potential application in pharmaceutical, cosmetic and leather industries, since their specific substrate is keratin, a fibrillar protein present in skin and its appendages.

Research is underway to further increase the enzymatic yield in a bioprocess by genetic modification of the fungal strain. In order to clone the keratinase from a not fully investigated filamentous fungus *D. microsporus* we intended to construct a genomic DNA library. The first, and the most important, step in the genomic DNA library

synthesis involves isolation of high-molecular-weight DNA. Until now, no protocol for *D. microsporus* has been described. Our aim was to develop a suitable protocol to obtain high-molecular-weight genomic DNA from the fungus, which could later be used to construct a genomic DNA library.

2. Experimental

2. 1. Enzyme and General Procedures

The restriction enzyme *Sau*3AI, T4 DNA ligase, *Bam*HI-predigested LambdaGEM-11 *Bam*HI arms were from Promega (Madison, WI, USA), a Gigapack III Gold Packaging Extract was from Stratagene (La Jolla, CA, USA) and DNase-free RNase, phenol equilibrated with TRIS buffer pH 8.5 were from Sigma (St. Louise, MO, USA). Basic procedures for DNA manipulations and horizontal agarose gel electrophoresis are described by Sambrook et al.[18] Other chemicals were DNase and RNase free.

2. 2. Microorganism and Cultivation

The keratinolytic filamentous fungus *Doratomyces microsporus*, strain MZKI B399[15] was obtained from the Culture Collection of The National Institute of Chemistry, Ljubljana, Slovenia. Spores were prepared by growing the fungus on agar slants at 30 °C for seven days. A sterilized complete medium, according to Benčina et al.,[19] in aliquots of 100 mL in 0.5 L Erlenmayer flasks was inoculated to give 10^9 spores L^{-1} of the medium and incubated for 20–22 h at 30 °C with shaking at 125 rpm (IS-200K, Kambič, Slovenia).[19, 20]

The fungal mycelium was harvested by filtration, rinsed with physiological solution (10 mM sodium phosphate buffer, 0.8% (w/v) NaCl, pH 6) and either frozen with liquid nitrogen or resuspended in KMC buffer pH 5.8 (1 M KCl, 50 mM $CaCl_2$, 20 mM MES).

2. 3. Disruption of Fungal Cell Wall

Different principles were used to disrupt the cell wall of the filamentous fungus.

(i) *Grinding mycelium in liquid nitrogen.* The harvested mycelium was washed with a physiological solution, dried between two sheets of filter paper and frozen under liquid nitrogen. The frozen mycelium was crushed in a mortar with pestle under liquid nitrogen to form a fine powder. Powdered mycelium was collected, weighed and the DNA extracted.

(ii) *Rapid freezing and thawing of mycelium.* The harvested mycelium was washed with a physiological solution. Then it was consequently rapidly frozen in liquid nitrogen followed by thawing in a water bath at 90 °C up to 10 times. During immersion of samples in water bath, they were gently mixed and as soon as the frozen content melted the samples were removed from the water bath and immersed in liquid nitrogen. Finally, the DNA was extracted.

(iii) *Enzymatic degradation of fungal cell wall.* Fungal protoplasts were prepared by resuspending the fresh mycelial pellets (2 g) in 20 mL of KMC buffer with 100 mg of lysing enzymes Glucanex (NovoNordisk, Bagsværd, Denmark) by mixing and then incubating for 2 h at 30 °C. During the two-hour process occasional mixing of the mycelium was performed to resuspend it in KMC buffer. Upon completion of hydrolysis, the obtained protoplasts were separated from the remaining mycelia by filtration through a nylon mesh (square side size between 0.1 to 0.2 mm). Protoplasts were collected from the filtrate by centrifugation at 3500 × *g* for 10 minutes (Andreas Hettich, Tuttlingen, Germany, rotor 1617) and dissolved in 0.2 mL of KMC buffer.

2. 4. Isolation of DNA

Three different procedures were tested as follows.

(i) *Isolation of genomic DNA with the commercial reagent DNAzol.* 0.5 g of the pulverised mycelium obtained after grinding in liquid nitrogen, or 0.2 mL of the protoplasts obtained after enzymatic treatment were carefully mixed with 5 mL of DNAzol (Life Technologies, Gaithersburg, MD, USA). After centrifugation at 10,000 rpm for 10 minutes an upper water phase containing the DNA was collected and DNA was precipitated with isopropanol and dissolved in either TE buffer (10 mM Tris, 1 mM EDTA, pH 7.6) or 8 mM NaOH.

(ii) *Isolation of genomic DNA with phenol extraction buffer.*[7] 1 g of the pulverised mycelium or 0.2 mL of the protoplasts was mixed with 2 mL of a phenol extraction buffer. The phenol extraction buffer contained 1.5 volume phenol equilibrated with Tris pH 8.0, 1 volume triisopropyl naphtalene sulphonic acid (Acros Organics, Belgium) (0.02 g mL^{-1}), 1 volume p-amino-salicylic acid (0.12 g mL^{-1}) and 0.5 volume 5 x RNB buffer (1 M Tris; 1.3 M NaCl, 0.25 M EGTA pH 8.5). The buffer was heated to 55 °C. The mycelium and the phenol-extraction buffer were mixed by gently inverting the tube for 1 min, 0.5 mL chloroform was added, and then mixed again for another 1 min. The aqueous phase was separated from the organic phase by centrifugation at 10,000 rpm (Centrifuge 5415R, Eppendorf, Germany) at room temperature for 10 minutes. The upper phase with DNA was additionally treated with 1 volume of chloroform to remove remnants of phenol. Chloroform was removed by centrifugation (Figure 1). The DNA was precipitated with isopropanol.

(iii) *Salting-out method.*[12] 2 g of the pulverized mycelium were resuspended in 5 ml SDS buffer (final concentration 1% (w/v) SDS), the proteinase K (0.25 g L^{-1} of buffer containing 75 mM NaCl, 25 mM EDTA pH 8, 20 mM Tris pH 7.5) was added and the mixture was incubated for 2 h at 55 ºC. After incubation, NaCl to a final

concentration of 1.25 M and 1 volume of chloroform were added. The precipitated proteins, polysaccharides and cell wall remnants were removed by centrifugation for 15 minutes at 6,000 rpm (Andreas Hettich, Tuttlingen, Germany, rotor 1615). The clear upper phase containing the DNA was collected and the DNA was precipitated as described below.

2. 5. DNA Precipitation

The DNA was precipitated from the aqueous solution with 0.7 volume of isopropanol at room temperature and collected either onto a sealed Pasteur pipette or, if the DNA pellet was not visible, with centrifugation at 4° C and at 12,000 *g* (Centrifuge 5415R, Eppendorf, Germany) for 15 minutes. The DNA pellet was then washed with 75% (v/v) ethanol and collected as described above. To remove RNA, the DNA was treated with DNase-free RNAase (10 mg L^{-1}). The air dried DNA was resuspended in TE buffer (10 mM Tris, 1 mM EDTA, pH 7.6) containing DNase-free RNase. If necessary, an additional step of phenol/chloroform extraction was performed (Figure 1), followed by DNA precipitation with 0.1 volume sodium acetate buffer (3 M sodium acetate, pH 5.2) and 2 volumes of 96% (v/v) ice-cold ethanol.

2. 6. Analysis of DNA by Pulsed-Field Electrophoresis

The size and quality of the DNA were determined using pulsed field electrophoresis with 1.2% (w/v) agarose gel in TBE buffer (45 mM Tris, 45 mM boric acid, 1 mM EDTA). The electrophoresis was performed at 120 V to 180 V, overnight, with a pulse of 5 s. The DNA was visualised by staining the gel in ethidium bromide (0.2 mg L^{-1}) for 30 minutes.

2. 7. Genomic DNA Library Construction

A genomic DNA library in the form of bacteriophages was constructed according to the manufacturers' protocol (Promega, Madison, WI, USA). The restriction enzyme *Sau*3AI was used for partial digestion of high-molecular-weight DNA. The concentration of the restriction enzyme *Sau*3AI appropriate for generating 9 to 23 kilo base pair (kbp) DNA fragments was determined as described in the manufacturers' protocol (Promega, Madison, WI, USA). Briefly, a defined amount (1 µg) of the high-molecular-weight DNA was mixed with serial dilutions of *Sau*3AI, from 1 to 0.0033 U per µg DNA and incubated at 37 °C for 30 min. The reaction was stopped with the addition of EDTA at pH 8.0 (10 mM final concentration). Products of partially digested DNA were precipitated and analysed by pulse-field electrophoresis.

For the large scale partial digestion of high-molecular-weight DNA, for 100 µg of DNA, a 0.0125 U *Sau*3AI restriction enzyme per µg DNA was used at 37 °C for 30 minutes in a final volume of 5 mL. The concentration of the restriction enzyme (0.0125 U per µg DNA) was half the amount determined in the optimization experiment (0.025 U per µg DNA). The reaction was stopped with 0.1 mL 0.5 M EDTA pH 8.0. The DNA from the reaction mixture was extracted with phenol/chloroform and precipitated. The DNA fragments in the size range between 9 and 23 kbp were isolated by sucrose gradient centrifugation. [21]

The DNA fragments of the appropriate size were ligated with dephosphorylated *Bam*HI-predigested EMBL4 lambda arms (Promega). The molar ratio of the lambda arms to the genomic insert varied from 1 : 1 to 1 : 3. The ligation mixture was packaged into phages *in vitro* according to the manufacturers' protocol (Gigapack III Gold Packaging Extract, Stratagene, La Jolla, CA).

After the library was packed it was amplified by secondary transfection of bacteria *E. coli* LE392 and stored. The freshly packaged and titrated library was adsorbed to bacteria *E. coli* LE392. The mixture was plated at a high density and allowed to grow until the plaques were still subconfluent. The phages were eluted from the plate by incubation overnight with a phage buffer (0.01% gelatin, 50 mM Tris pH 7.5, 100 mM NaCl, 8 mM $MgSO_4$) and stored at –70 °C.

2. 8. Separation of DNA Fragments by Sucrose Density Gradient

The high-molecular-weight DNA, partially digested with *Sau*3AI, was separated according to size using sucrose gradient centrifugation.[18] The DNA solution [75 µg of DNA in 500 µL STE buffer (1 M NaCl, 20 mM Tris, 5 mM EDTA pH 8.0)] was heated to 65 °C for 5 min to dissociate the aggregated DNA fragments. It was then loaded onto a high-salt sucrose gradient [10–40% (w/v) sucrose gradient, 1 M NaCl, 20 mM Tris, 5 mM EDTA pH 8.0] and centrifuged at 113,000 × *g* for 22 hours at 20 °C (Beckman L7-55, rotor SW-28). Fractions (1.5 mL) were collected and the DNA size was analysed using pulsed-field electrophoresis. Fractions containing the DNA fragments with a size between 9 and 23 kbp were pooled, dialysed against TE buffer and precipitated with ethanol.

3. Results and Discussion

For the construction of a representative genomic DNA library the quality of the starting material, the isolated genomic DNA, is of crucial importance. To obtain the high-molecular-weight genomic DNA with a size above 100 kbp, shear forces during the isolation procedure should be minimised. Due to the specificity of fungal mycelial cells, a procedure for DNA isolation should be optimised to achieve the required length of DNA for library synthesis. The most commonly used method[7] which

includes grinding, phenol and chloroform extraction, and DNA precipitation is tedious and made up of several phases. On the other hand, this procedure was confirmed to give the best result[7] for the isolation of genomic DNA from *Aspergillus sp*.

3. 1. Cell Wall Disruption

The fungal cell wall represents a rigid, mechanical barrier on the surface of the protoplast and in such a way it determines the shape of the cell. It is composed mainly of structural polysaccharides. Two basic principles to remove the fungal cell wall are: (i) enzymatic degradation[22] and (ii) mechanical approach.[7,8,23] Therefore, in the first stage we tested the efficiency of three different approaches: freezing the young mycelium in liquid nitrogen and then grinding;[7] sequential rapid freezing and thawing of the mycelium;[8] generating protoplast with lytic enzymes.[22]

The efficient removal of cell wall is a prerequisite for good DNA yield and its length. It was expected that the last two methods mentioned above, namely, rapid freezing and thawing and generating protoplasts by enzymatic hydrolysis of the cell wall, would be less damaging for the DNA and would give better results since they include less friction and shear stress on the DNA molecule. Surprisingly, the longest DNA with 100–150 kbp and the best yield giving 125 μg DNA per g of mycelium was obtained by grinding in liquid nitrogen (Table 1). Therefore, this method was chosen for the first stage of genomic DNA isolation from *D. microsporus*.

A low DNA yield and poor DNA quality obtained with the other two methods could be assigned to long processing times from collecting mycelium and to extraction. The method with sequential rapid freezing and thawing yielded 67 μg DNA per g mycelium when mycelium was treated with 10 sequential rapid freeze/thaw cycles. When less than 10 cycles were performed DNA yield was lower. An increase in the number of cycles did not improve DNA yield and DNA quality. Nucleases were not inhibited during the first stage, which might be an explanation for low DNA yield and insufficient length. With the method used for cell wall disruption with lytic enzymes, we obtained a very low DNA yield. This could be explained with the low efficiency of protoplast formation. Not all of the protoplasts stay intact which leads to leaking out cellular components into the lysis buffer.

Table 1. Amount of DNA and its length isolated with phenol-extraction buffer after three different methods of cell wall removal.

Cell wall disruption method	Amount of DNA [μg per g mycelium]	Length [kbp]
Grinding in liquid nitrogen	125	100–150
Sequential freezing and thawing	67	~ 50
Enzymatic hydrolysis	7	< 50

Unfortunately, cell wall disruption by grinding in liquid nitrogen is a labour-demanding method and is not a first choice method if speed and high throughput are required.

3. 2. Extraction of High-Molecular-Weight DNA

In the second phase the removal of contaminants such as soluble proteins and polysaccharides is required. Three different procedures were tested: (i) a method adapted from protocols for filamentous fungal species

CELL WALL DISRUPTION

1. Grinding of washed and frozen mycelium in liquid nitrogen to generate fine powder.

DNA ISOLATION

2. Protein and polysaccharide precipitation with phenol extraction buffer (2 mL per 1 g mycelium); gentle mixing 5 minutes; incubation 10 minutes, 55 °C.
3. Addition of chloroform (0.5 mL); gentle mixing 1 minute; centrifugation (10,000 rpm, room temperature; 10 minutes).
4. Collection of upper water phase containing DNA; addition of chloroform (1 volume); gentle mixing 1 minute; centrifugation (10,000 rpm, 4 °C; 5 minutes).
5. Collection of upper water-DNA phase; DNA precipitation with isopropanol (0.7 volume); gentle mixing; centrifugation (10,000 rpm, 4 °C; 10 minutes).
6. Dissolving the DNA pellet in TE buffer with RNase.

RE-PURIFICATION

7. Addition of phenol : chloroform 1 : 1 (1 volume); gentle mixing 1 minute; centrifugation (10,000 rpm, 4 °C, 10 minutes).
8. Collection of upper water-DNA phase; addition of chloroform (1 volume); gentle mixing 1 minute; centrifugation (10,000 rpm, 4° C, 5 minutes); repeated twice.
9. DNA precipitation with sodium acetate (0.1 volume) and ethanol (2 volumes); centrifugation (10,000 rpm, 4 °C; 10 minutes).
10. Washing the DNA pellet with 70% ethanol (1 volume); centrifugation (10,000 rpm, 4 °C, 5 minutes).
11. Dissolving the DNA pellet in TE buffer.

Figure 1. Flowchart of high-molecular-weight DNA isolation method for the fungus *D. microsporus*.

Table 2. Amount of DNA and its length isolated when using different extraction methods after cell wall removal by grinding in liquid nitrogen.

Extraction agent	Additional purification with phenol: chloroform (1:1)	DNA concentration [μg per g mycelium]	Length [kbp]
DNazol	–	30	50–100
	+	2	< 50
DNA extraction buffer	–	125	100–150
	+	50	100–150

Aspergillus sp., using a phenol-extraction buffer[7] (ii) a rapid method for DNA isolation with a commercial product DNAzol and (iii) a salting-out method that included proteinase K to remove proteins giving very good results with filamentous bacteria *Streptomyces sp*.[6,12,13,23] Additionally, we have tested extraction by phenol alone,[18] however, only a small amount of DNA with molecular length less than 10 kbp was obtained. According to the results (Table 2), DNA extraction with the phenol-extraction buffer yielded the best DNA quality with a suitable length of 100–150 kbp to proceed in the genomic DNA library synthesis. Isolation of DNA with reagent DNazol is rapid and simple. However, precipitated DNA poorly dissolved in TE buffer. On the other hand, when dissolved in sodium hydroxide as recommended by the manufacturer, the DNA was damaged as shown. In addition, the length of DNA was around 50 kbp, which is not good enough for synthesis of a bacteriophage DNA library. The yield of DNA obtained with the salting-out method was 20 μg of DNA per g of mycelium, with a length around 50 kbp.

Constituents of a phenol-extraction buffer are more lipophilic than sodium dodecyl sulphate. The phenol-extraction buffer contains EGTA and p-amino-salicylic acid to chelate metal ions and indirectly inhibit DNases,[11] and a detergent to improve precipitation of polysaccharides and proteins. The components of phenol-extraction buffer are fully dissolved at pH above 8.0 and a temperature higher than 50 °C which ensures correct buffer pH and temperature.

Based on the overall results a final protocol was designed, which is depicted in Figure 1.

Finally, the isolated DNA was detected by pulse-field electrophoresis as shown in Figure 2A.

3. 3. Construction of Genomic DNA Library

The quality of the obtained genomic DNA and restriction enzymes used for synthesis of a DNA library are crucial for a successful result. The DNA must be of high molecular weight. A useful insert fragment must have both ends generated by restriction enzymes to be able to ligate into the chosen vector. In order to generate a majority of the random fragments with two good ends, the starting DNA material must be on average at least three times longer (> 70 kbp) than the insert of about 9–23 kbp to be used in the cloning procedure.

The isolated high-molecular-weight genomic DNA of *D. microsporus* was partially digested with *Sau*3AI restriction enzyme (Fig. 2B). With the use of the optimal dilution (1 : 1200) of the restriction enzyme the fractions with DNA fragment size between 9 and 23 kbp (Fig. 3A) were isolated by sucrose gradient centrifugation (Fig. 3B). A size separation of DNA fragments in sucrose gradient is time consuming but has high size resolution between 5 to 60 kbp DNA. Isolation of suitable fragments of the fungal genomic DNA is shown in Fig. 3C.

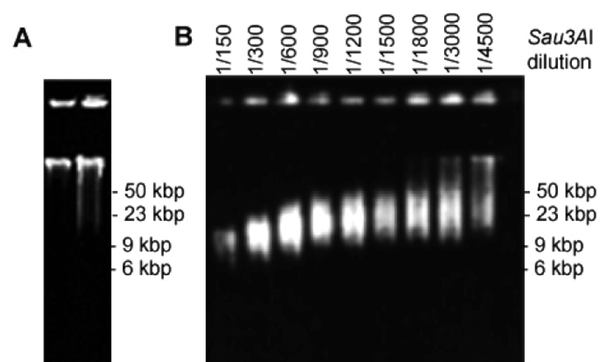

Figure 2. [A] Pulse-field electrophoresis of the isolated high-molecular-weight genomic DNA which was used as starting material for optimization of partial digestion. Procedure depicted in Figure 1 was used for DNA isolation. [B] Partial digestion of high-molecular-weight DNA of *D. microsporus*. Pulse-field electrophoresis of partially digested starting material after using different dilutions of the restriction enzyme *Sau*3AI (from 1/150 to 1/4500).

Ligation of the purified fragments with a size in the range between 9 and 23 kbp into *Bam*HI-predigested lambda arms (9 kbp and 20 kbp) is shown in Fig. 4. The appearance of a ligation product with a size of 50 kbp fragment, which is required for bacteriophage packaging, is clearly visible.

The obtained ligation mixture was *in vitro* packaged into bacteriophage particles and transfected into bacteria *Escherichia coli* LE392. After lysis of bacterial cells, bacteriophages were collected. A bacteriophage titer of the primary genomic DNA library was 10^5 plaque-forming units (PFU) that presented a 1.6 Gbp DNA.

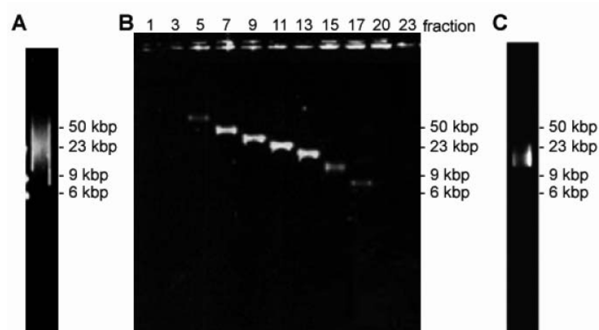

Figure 3. [A] Analysis of preparative partially digested DNA (an aliquot) resolved by pulse-field electrophoresis. Preparative partial digestion of the high-molecular-weight DNA was performed with the restriction enzyme *Sau*3AI (dilution 1/1200 which corresponds to 0,0125 U *Sau3A*I per mg of DNA). A band of partially digested DNA with sizes from 9 to 50 kbp was determined with the electrophoresis. [B] Fractions of the partially digested DNA after centrifugation in sucrose gradient. [C] Collected fractions of partially digested DNA suitable for construction of bacteriophage genomic DNA library. Fractions with sizes between 9 and 23 kbp (fractions from 11 to 15 see Fig. 3B) were collected and an aliquot was analyzed by pulse-field electrophoresis.

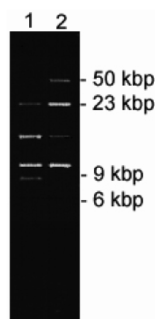

Figure 4. Ligation of partially digested genomic DNA into bacteriophage arms LambdaGEM-11 *Bam*HI arms. Legend: control without ligase [1] and ligation mixture [2].

To amplify the primary genomic DNA library from *D. microsporus* an additional step of *E. coli* transfection was introduced and a secondary bacteriophage stock was collected. A bacteriophage titer of secondary genomic DNA library from *D. microsporus* was 3 x 10^8 PFU, which accounted for 4500 Gbp DNA. Since the size of the genome for *D. microsporus* is not known an exact coverage of genome with the genomic library could not be determined. If we assume that the size of the *D. microsporus* genome is in the range of genome sizes of *Aspergillus nidulans* (30 Mbp), *Magnaporthe grisea* (39 Mbp) or *Neurospora crassa* (39 Mbp) the coverage is approximately 100 times. Approximately 10,000 independent clones would need to be screened to isolate a particular sequence with a probability of 99%[21] from this bacteriophage DNA library.

4. Conclusions

For isolation of high-molecular-weight DNA from the filamentous fungus *Doratomyces microsporus*, the method using phenol extraction buffer gave the largest quantity and the best quality of DNA regarding its length.

For the cell wall removal, grinding of mycelium in liquid nitrogen was preferable to disruption of the cell wall with lytic enzymes and to the method using sequential rapid freezing and thawing.

In the extraction step, the phenol-extraction buffer proved to be the reagent of choice over DNAzol and the salting-out method.

To obtain pure DNA, an extra precipitation of DNA had to be performed.

Using the complete protocol, including cell wall disruption, DNA isolation and re-purification, sufficiently long DNA fragments from 100 to 150 kbp could be obtained, which were suitable for genome library construction.

Using the optimised protocol a genomic DNA library of the filamentous fungus *D. microsporus* could be prepared with approx. 100 times genome coverage.

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Povzetek

Razvili in optimirali smo protokol za izolacijo DNA filamentozne glive in pripravili gensko knjižnico. Poznane so številne metode priprave genomske DNA: metode, ki omogočajo obdelavo velikega števila vzorcev in metode, ki omogočajo izolacijo visoko kvalitetne DNA, kar je še posebej pomembno za sintezo genomske DNA knjižnice. Izolacija DNA visoke molekulske mase iz glive predstavlja precejšen izziv, ker se sestava celične stene in celična vsebina razlikujejo med posameznimi vrstami gliv. Opisan je optimiziran postopek izolacije dolge visoko kvalitetne DNA keratinolitične filamentozne glive *Doratomyces microsporus*, sev MZKI B399, ki smo ga primerjali z drugimi metodami. Kot najprimernejša metoda za izolacijo DNA se je izkazalo trenje micelija v tekočem dušiku v kombinaciji z odstranitvijo proteinov in polisaharidov iz raztopine DNA z ekstrakcijskim pufrom in obarjanjem DNA z izopropanolom. Tako pripravljeno DNA smo uporabili za pripravo genomske DNA knjižnice glive.