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EVALUATION OF THE EFFECTIVENESS OF USING A UNIVERSAL METHOD FOR ISOLATING GENOMIC dsDNA BY SALTING OUT TECHNIQUE ACCORDING TO THE S.M. ALJANABI AND I. MARTINEZ PROTOCOL FOR YEAST *SACCHAROMYCES CEREVISIAE*

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Abstract
This work evaluated the possibility of using the universal salting out genomic dsDNA isolation method according to the S.M. Aljanabi and I. Martinez protocol from yeast cells of *Saccharomyces cerevisiae* also for possible PCR-based applications. Our modification of this protocol adapted it for the extraction of DNA from the liquid culture of *S. cerevisiae* yeast colonies/cells. This protocol did not require any hazardous chemicals or specific conditions and was specially developed for the simultaneous processing of many samples. Prepared suspension liquid of 100 µL according to the McFarland Standard method corresponds to $1.5x10^8$ cells per volume unit. Turbidity standards provide the opportunity to visually determine the number of cells per unit volume by turbidity. For fungi, a culture with a value of 0.5 McFarland Standard contains 30 times less microbial cells than enterobacteria. Approximately 400 ng/µL of total genomic dsDNA (*S. cerevisiae*) can be isolated from 1.5×10^8 *S. cerevisiae* cells. The isolated dsDNA had good quality and purity (A_{260}/A_{280} ~1.8-2.0 ±0.2-0.3 and A_{260}/A_{230} in the range of 2.0-2.2 ±0.2-0.3). Isolated dsDNA corresponds to high molecular weight dsDNA with high levels of nucleic acids in 1 µL of solution. This modified DNA extraction technique is as a rapid and universal low-cost genomic dsDNA isolation protocol that is suitable for yeast cell liquid cultures.

Keywords: DNA concentration, DNA electrophoresis, DNA extraction, *Saccharomyces cerevisiae*.

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INTRODUCTION

Saccharomyces cerevisiae, also known by its common names, baker's or brewer's yeast is a unicellular microscopic (5–10 microns in diameter) fungi (yeast) from the class of *Saccharomycetes*, widely used in the production of alcoholic and bakery products, as well as in scientific research (Pereira et al. 2021).

By May 1996, the first complete eukaryote genome sequence of a eukaryote – the budding yeast *Saccharomyces cerevisiae* was obtained.

Saccharomyces cerevisiae is considered a low-virulence human pathogen, so working with them does not require extreme precautions (Raghavan et al. 2019). Yeasts adapt well to many types of conditions and changing stressful environments (Dhar et al. 2013). Yeasts are ubiquitous, single-celled microorganisms belonging to the kingdom Fungi, with about 2000–2200 species described (Boekhout et al. 2022). Like all other eukaryotic organisms, the yeast cell carries its genetic information on chromosomes that are sequestered from other cellular constituents by a well-organized double membrane, which forms the nucleus (Taddei & Gasser 2012).

Yeasts of the genus *Saccharomyces* have been widely used as model objects which provide useful data, especially in cytology, biochemistry, and genetics (Botstein et al. 1997). Thus, the science of yeast, having been studied for more than half of the century, continues to develop rapidly in the 21st century (Botstein & Fink 2011).

Yeast cell walls are relatively rigid structures due to β(1,3)-glucans (Bzducha-Wróbel et al. 2014), which is the main obstacle for quick and easy lysis of yeast cells and therefore must be disrupted for efficient recovery of genomic DNA. Accustomed methods for obtaining genomic DNA from yeast cells use either enzymatic degradation or beating with glass beads, followed generally by lysis of cells with a detergent and extraction of genomic DNA with phenol-chloroform. However, these methods are time-consuming and relatively expensive making it inconvenient for the simultaneous handling of a large number of samples. Even the so-called rapid and efficient protocols require expensive or toxic reagents (Paterson et al. 1993, Harju et al. 2004, Borman et al. 2006). In addition, many papers have been published on genomic DNA extraction in yeast cells, for example, by the thermolysis buffer or CTAB method. These methods for dsDNA extraction from yeast cells and bacteria were more laborious, the yield of genomic DNA was relatively low and the results were poorly reproducible as well as the thermolysis buffer was not recommended for extracting DNA from fungal tissues because the cell walls of fungal tissues were more difficult to break with this method than those of mycelia (Zhang et al. 2010). For this reason, it is necessary to search for a more complex and optimized DNA extracting protocol to find out the critical components for effective genomic DNA extraction from yeast cells. There is evidence that this universal method of DNA extraction has previously been successfully used for isolation genomic DNA from animal tissues, plants, and insects for different applications (Drábková et al. 2002, Trzewik et al. 2016, Petjukevičs & Škute 2017, Oreha & Škute 2022).

Therefore, the present study aimed to test a universal dsDNA salting-out extraction method according to the Aljanabi and Martinez's (1997) protocol, as well as optimize and elucidate the critical aspects for the efficient extraction of dsDNA from yeast.

MATERIALS AND METHODS

Cultivation of *Saccharomyces cerevisiae*

1 g of yeast (*Saccharomyces cerevisiae*) was inoculated into 9 ml of BRAIN HEART INFUSION BROTH (BioMaxima, Poland). Test tubes were placed in a thermostat for 24 hours at a temperature of +25°C. Then 1 ml of the inoculum was sown on a dense agar medium Plate Count LabAGAR (BioMaxima, Poland) by surface method and cultivated at 25°C within 48 hours (Refrigerated Incubator FTC 90E). After 48 hours of

cultivation, the culture gave abundant growth, the growth of typical of *Saccharomyces cerevisiae*: white, rounded, the surface is smooth, convex, shiny, the edges are even, homogeneous structure, mucous consistency, size with a diameter of 1–3 mm. Drying the drop at room temperature (+20), the preparation was fixed in a flame. Staining was carried out by microscopy of isolated colonies, the most commonly used stains for visible light microscopy are methylene blue, safranin, Lugol's solution and tetrazolium chloride (Gram stain), immersion oil and examined with a 100x objective on a light microscope (Carl Zeiss stereomicroscope Axioscop 40).

Extraction of genomic dsDNA from yeasts

The salting-out method was used to isolate yeast genomic dsDNA (Aljanabi & Martinez 1997). An aliquot of 100 µL liquid yeast culture 10^8 cell/mL by 0.5 (McFarland Standard) was homogenized in 400 µl lysis buffer (0.4 M NaCl, 10mM Tris-HCl, pH 8.0 and 2mM EDTA, pH 8.0) and 40 µL of 20% SDS (Sigma-Aldrich CO., USA) and 8 µL of 20 mg/mL proteinase K (Promega, USA) were added. The homogenates were incubated at 65°C for 60 min in a water bath (WB4) and then 300 µL of 6 M NaCl was added to each sample. The suspensions were vortexed (BioVortex V1) thoroughly for 30 s at maximum speed and tubes were centrifuged (Eppendorf mini Spin plus) at full speed for 30 min. The upper aqueous layer was carefully transferred to clean centrifuge tubes and an equal volume of cold isopropanol (Enola, Latvia) was added to the DNA sample. The tubes were allowed to stand at -20°C for 60 min and centrifuged at $\geq 10{,}000 \times g$ for 20 min. The dsDNA pellets were washed with ice-cold 70% ethanol (Enola, Latvia) and centrifuged at $\geq 10{,}000 \times g$ for 20 min. The resulting dsDNA pellet was air-dried at room temperature and dissolved in 100 µL of TE (Tris-HCl 10 mM pH 7.6, EDTA 1 mM pH 8.0). The yeast dsDNA samples were stored at -18°C.

Qualitative and quantitative analysis of dsDNA by agarose electrophoresis and spectrophotometry

Agarose gel electrophoresis was used to estimate potential dsDNA degradation (Takahashi et al. 2022). dsDNA purity and concentration were determined spectrophotometrically (Shimadzu BioSpec-nano). The relative absorbance readings were investigated for contamination by carbohydrates, peptides, aromatic compounds, humic acids and phenol (A_{260}/A_{230}) and proteins (A_{260}/A_{280}) using spectrophotometry (Budel et al. 2022, Skute et al. 2020). Analysis of genomic dsDNA integrity was confirmed by horizontal electrophoresis on a 1.8% (w/v) agarose gel (Consort EV243): 1st step – 15 min 60 V, 2nd step – 2 h 90 V and stained with ethidium bromide (20 µL per 100 mL of buffer). The agarose gel (Cleaver Scientific Ltd.) was analyzed and visualized on a 302-nm UV light transilluminator (multispectral imaging system UVP-800).

Statistical analysis

The data were expressed as the mean±standard deviation and were analysed statistically using MS Excel 2019.

RESULTS AND DISCUSSION

Extracting dsDNA from yeast cells has been the focus of many studies as it marks a crucial step for any kind of further molecular investigations (Ligozzi & Fontana 2003, Harju et al. 2004, Lõoke et al. 2011). Previous studies have described also different dsDNA extraction procedures in yeast cells (Tapia-Tussell, 2006, Blount et al. 2016).

The isolation and purification of genomic dsDNA from *Saccharomyces cerevisiae* compared to the methods of genomic dsDNA extraction from animal and plant tissue are laborious and time-consuming or generate poor yields, particularly during extensive screening procedures in which many samples are prepared (Blount et al. 2016). The extraction using standard cell lysis proce-

dures appeared to be more efficient in producing a high concentration of genomic dsDNA than boiling and freeze-thawing extraction procedures. This method did not require certain conditions, hazardous chemicals and was specially developed for the simultaneous analysis of many samples.

A prepared suspension according to 0.5 McFarland Standard is usually used in extracting DNA from yeast, therefore, in the procedure for Aljanabi and Martinez (1997) we did not use the weight of the sample, but a pre-homogenized suspension before DNA isolation. Qualitative and concentrated DNA was obtained.

dsDNA quality was characterized in terms of purity using UV spectroscopy (Shimadzu, BioSpec-nano), where the A_{260}/A_{280} and A_{260}/A_{230} ratios are indicators of different contaminants, and intactness was evaluated using agarose gel electrophoresis. The concentration of purified genomic dsDNA in yeast cells suspensions ranged from 315 to 563 ng/µL. dsDNA purity and quantity were measured using UV spectroscopy, where the A_{260}/A_{280} and A_{260}/A_{230} ratios are indicators of different contaminants on dsDNA samples data are shown in Figure 1, Figure 2. The isolated dsDNA showed good performance in terms of dsDNA quality and dsDNA purity: A_{260}/A_{280} ~1.8-2.0±0.2-0.3 and A_{260}/A_{230} in the range of 2.0-2.2±0.2-0.3 data are shown in Figure 3, indicating low amounts of contaminating proteins and other organic contaminants, such as phenols and carbohydrates (Tab. 1) (Kopecká et al. 2014).

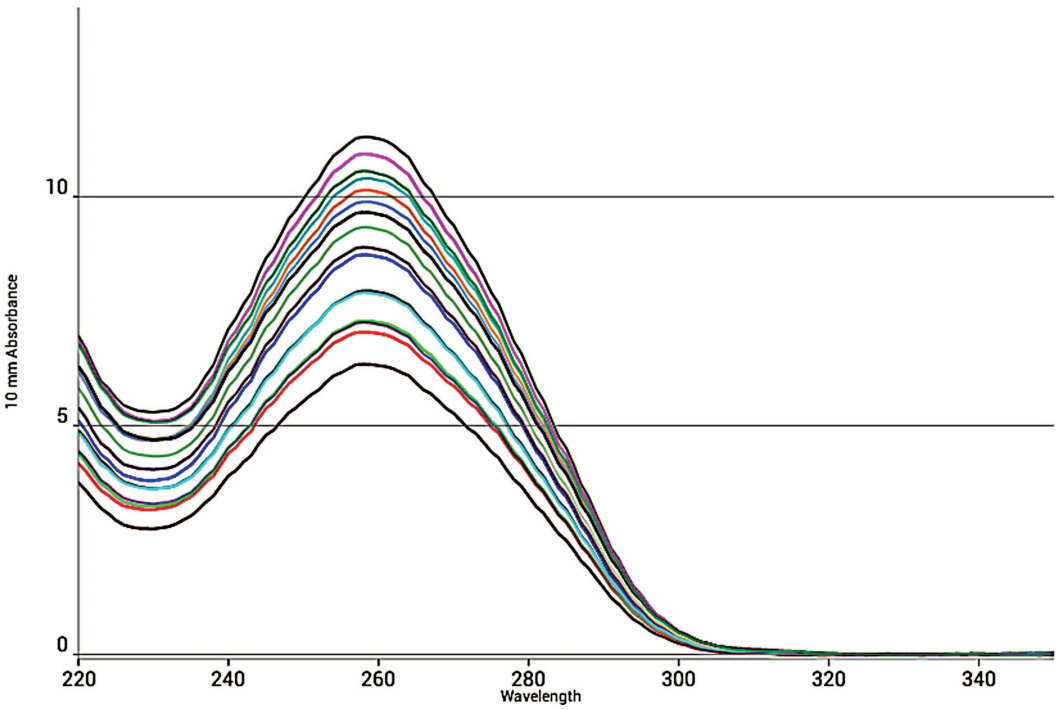

Figure 1. UV-Vis Spectrophotometry, scan profile of extracted *Saccharomyces cerevisiae* dsDNA.

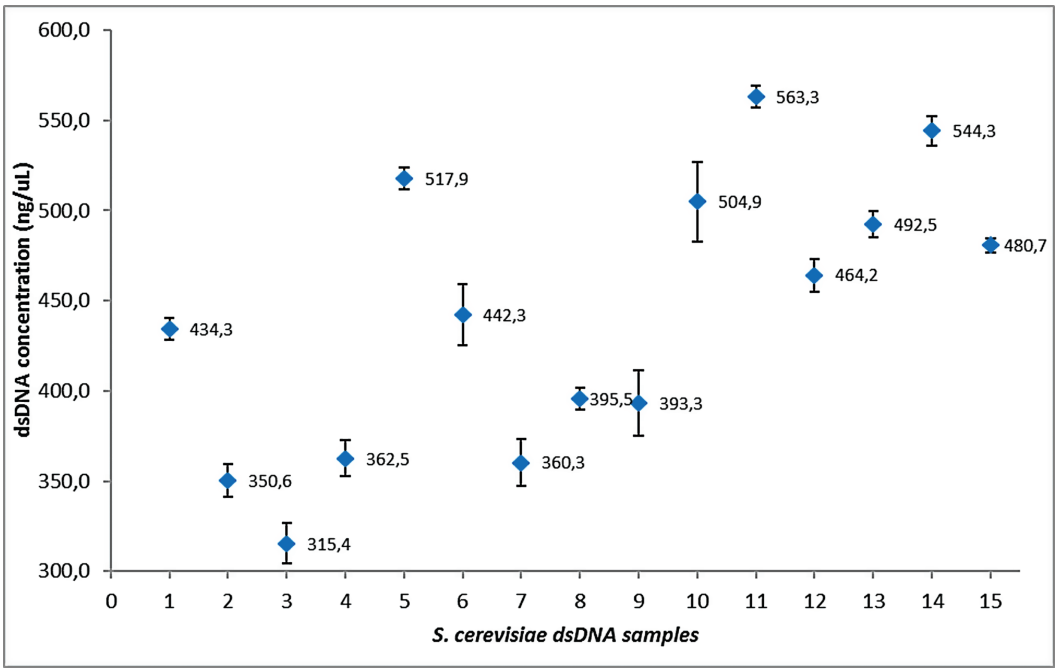

Figure 2. Quantification of extracted *Saccharomyces cerevisiae* DNA by UV-Vis Spectrophotometry (DNA samples: 1–15).

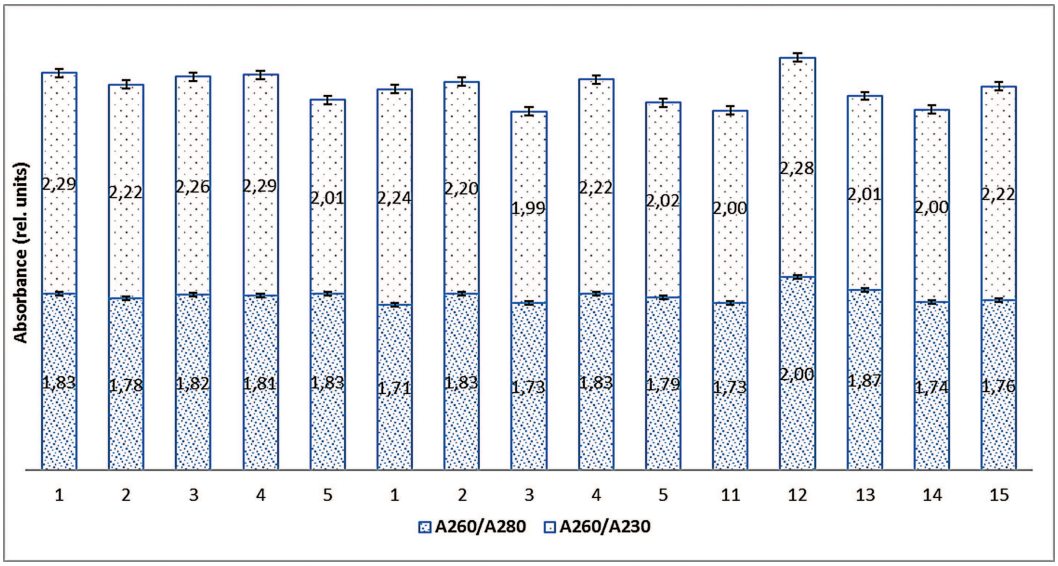

Figure 3. A_{260}/A_{280} and A_{260}/A_{230} Ratios of dsDNA isolated from *Saccharomyces cerevisiae* using the S.M. Aljanabi and I. Martinez universal protocol.

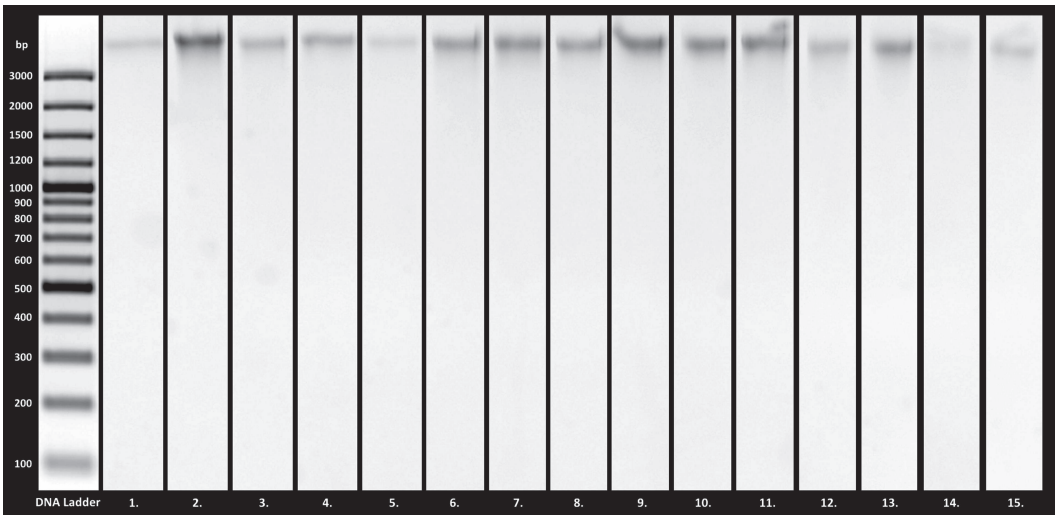

Figure 4. Visualisation of isolated yeast DNA (*Saccharomyces cerevisiae*) using the S.M. Aljanabi and I. Martinez universal protocol in 1.8% Agarose gel by horizontal electrophoresis. DNA size marker/Ladder: Gene Ruler 100bp. Plus DNA Ladder (Thermo Scientific).

Table 1. Comparison of the methods of DNA isolation from yeasts. Explanations: * Time required per samples, ** SEM (Salting-Out Extraction Method).

Extraction method	Time (min)*	Sample vol.	Yield (ng/µL)	Ratio 260/280	Ratio 260/230
Phenol/chloroform method	210 + overnight incubation	100 µL	6075	2.01	1.62
Kit A (Lyticase treatment, silica columns)	130 + overnight incubation	100 µL	598.50	1.76	0.75
Kit B (Lyticase treatment, silica columns)	110	100 µL	282.75	1.84	0.86
Kit C (Mechanical and chemical cell lysis, silica columns)	140 + overnight incubation	100 µL	45	0.53	0.04
SEM**	115 + overnight incubation	100 µL	400	1.9	2.1

Furthermore, we evaluated the integrity and fragmentation of extracted genomic dsDNA by performing agarose gel electrophoresis, which showed high-molecular-weight genomic dsDNA in lines 1–15 data are shown in Figure 4. It can be seen that DNA from all samples was high-molecular.

Our modification of genomic dsDNA isolation method according to S.M. Aljanabi and I. Martinez protocol implied being used for the cell lysis liquid culture *S. cerevisiae* yeast colonies/cells. In summary, the observations suggest that the protocol described by S.M. Aljanabi and I. Martinez is useful for the rapid and successful extraction of genomic dsDNA from yeast species and the yield and quality of the dsDNAs obtained allow for their use in different molecular techniques in genetics and molecular biology.

CONCLUSIONS

This study analyzed the possibility of using the universal method performed by S.M. Aljanabi and I. Martinez protocol for genomic dsDNA isolation from yeasts. After dsDNA isolation by this universal method, dsDNA was checked spectrophotometrically for quality level and contamination by phenolic compounds, proteins, and carbohydrates. dsDNA fragmentation and molecule integrity were checked by fractionation in 1.8% agarose gel. All these manipulations with isolated samples of genomic dsDNA from yeast (*Saccharomyces cerevisiae*) confirmed the efficiency of this universal technique and showed good performance. dsDNA samples were pure, not fragmented. The dsDNA was high molecular weight, with a high concentration of nucleic acids per 1 microliter of sample. Thus, S.M. Aljanabi and I. Martinez protocol can be considered suitable for isolating high-quality dsDNA from yeast.

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