Electroinduced release of low molecular components, total protein and β-galactosidase from *Kluyveromyces lactis* 1470

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Introduction

Kluyveromyces yeast genus is of considerable interest in biotechnology industry. This yeast genus is an important source of the enzyme β-galactosidase (EC.3.2.1.23), which is responsible for hydrolyzing lactose to its comprising monomers – namely glucose and galactose, thus finding application for the enzyme in food industry to process products intended for people with lactose intolerance.

An alternative method that achieves cellular permeabilization and lysis is treatment of cell suspensions with pulsed electric field with high intensity, which leads to the formation of pores in the cell membrane - electropermeabilization/electroporation. It has been shown that treatment of cell suspensions with pulsed electric field leads to efficient release of low molecular compounds such as aminoacids, antioxidants and nucleotides, as well as to the release of proteins, including cytoplasmic enzymes with preserved enzymatic activity.

Aim of the work

To investigate the applicability of using of pulsed electric fields (PEF) as an alternative method for selective extraction of low molecular weight intracellular components, total protein and the enzyme β-galactosidase in a significantly purified form from *K. lactis* 1470.

Materials and methods

- **Yeast strains***: Kluyveromyces lactis 1470*
- **Cultivation:** UPL; 48 h at 30°C
- **Cells:** washed and diluted in distilled water - 100 mg wet weight/ml (concentration during treatment)
- **Conductivity** of the cell suspension before treatment - 150 µS/cm
- **Electroporation:** generators of rectangular pulses
  - ***In batch treatment*** - P4000 (Cytopulse), standard Al electrodes 2 mm distance
  - ***In flow treatment*** - Hydropulse mini (Germany), chamber with stainless steel electrodes, 3 mm distance
- **After electrical treatment:** cells were incubated in water or potassium phosphate buffers (PPB) at room temperature for 5 min - 20 h
- **Determination of proteins:** Bradford assay
- **Determination of β-galactosidase activity:** using o-nitrophenyl-P-D-galactoside as substrate
- **Total antioxidant activity:** TAEC (trolox equivalent antioxidant capacity)- method

Results

Equal efficiency – batch and flow mode

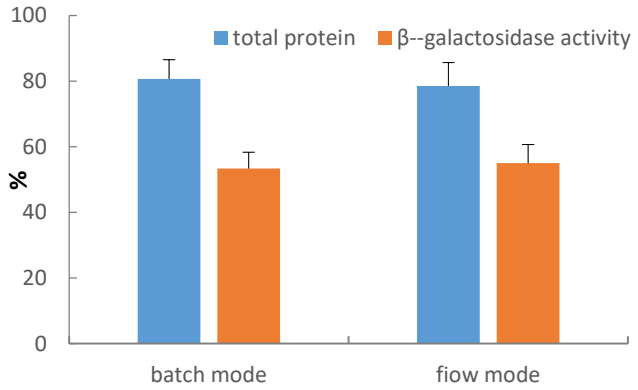


Fig.1. Release of total protein and ß-galactosidase from *K. lactis* 1470

Optimal conditions of electric treatment: 1) Batch mode: 10 pulses, 2 Hz, 1 ms duration, intensity of 5 – 5.125 kV/cm; 2) Flow mode: 10 pulses, 5 Hz, 1 ms duration, intensity of 4.67 – 4.83 kV/cm, flow rate 9 ml/min; Incubation: 50 mg/ml ww; 125 mM PPB pH 8.5, 5 mM DTT, 20h

Yeast cell wall - a barrier to the release of large intracellular molecules from permeabilized cells

DTT is reducing agent that breaks disulphide linkages in the cell wall and increases its permeability

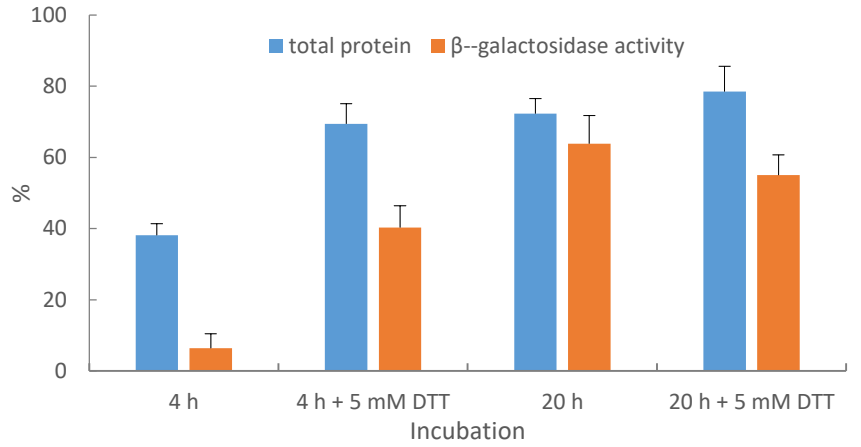
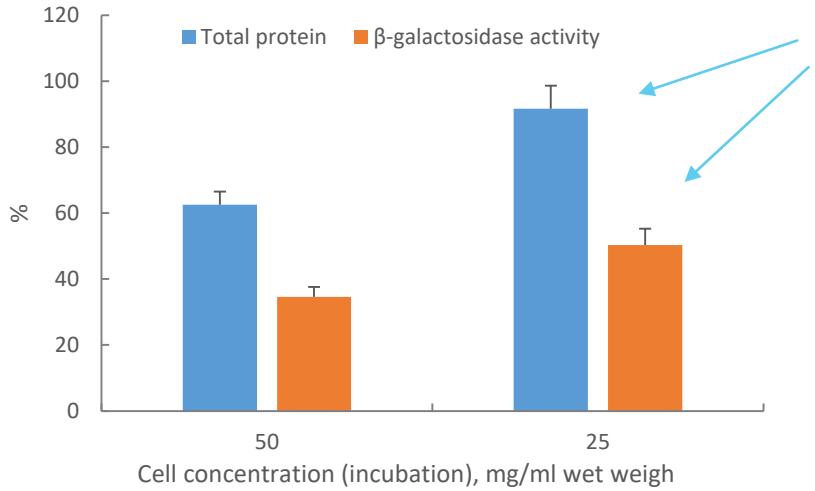


Fig. 2. Effect of DTT on total protein and ß-galactosidase release

Electrical conditions: 10 pulses (5 Hz), 1 ms duration; intensity of 5 kV/cm; Flow rate 9 ml/min (Flow mode); Incubation: 50 mg/ml ww, 125 mM PPB pH 8.5



92% protein;
50% ß-galactosidase activity
(four times dilution)

Fig.3. Influence of cell concentration during incubation.

Electrical conditions: 10 pulses (2 Hz), 1 ms duration, intensity of 5 kV/cm (batch mode)

Incubation: 125 mM PPB pH 8.5, 5 mM DTT; 4h

Partial purification of ß-galactosidase - two-stage incubation

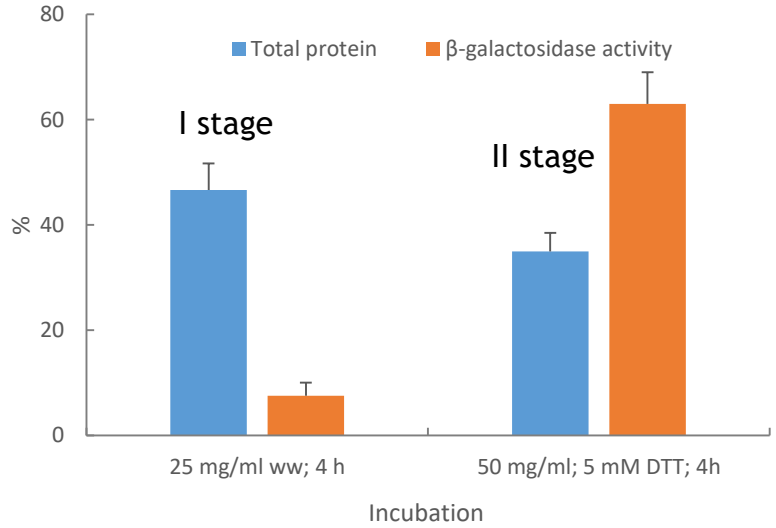


Fig. 4.Two-stage incubation of electropermeabilized cells *K. lactis* 1470

Electrical conditions: 10 pulses (2 Hz), 1 ms duration, intensity of 5 kV/cm (batch mode)

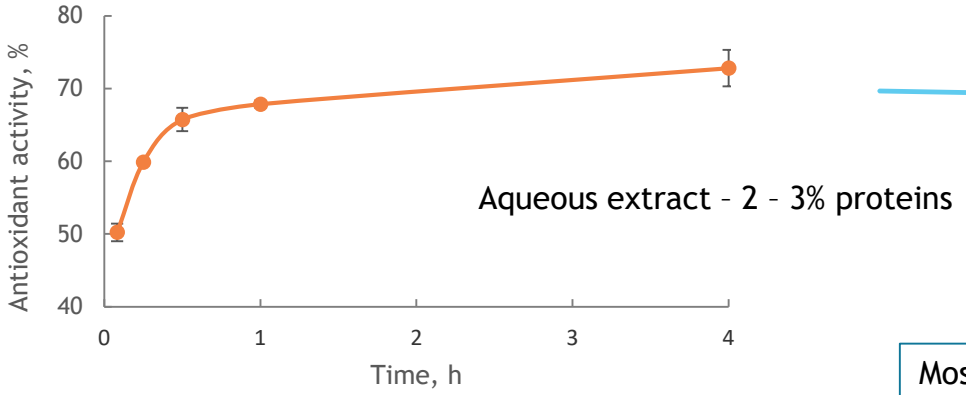
Incubation: I stage: 25 mg/ml ww, 125 mM PPB pH 8.5, 4 h

II stage: 50 mg/mo ww, 125 mM PPB pH 8.5, 5 mM DTT, 4h

Significant release of ß-galactosidase (II stage) - increased porosity of the cell wall

Treated cell suspension

1) Incubation in water



Aqueous extract – 2 – 3% proteins

2) The same cells were diluted in buffer

Most of antioxidant activity in yeast is due to a thermostable, non-enzymatic fraction

Fig. 5. Release kinetics of total antioxidant activity from *K. lactis* 1470

Electrical conditions: 10 pulses (5 Hz), 1 ms duration; intensity of 5 kV/cm; Flow rate: 9 ml/min (Flow mode); Incubation in water (without dilution)

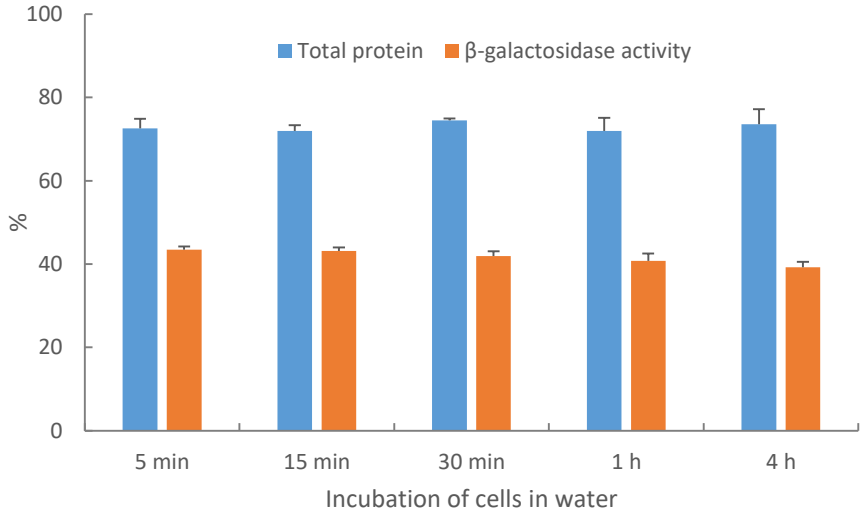


Fig. 6. Release of total protein and β-galactosidase from *K. lactis* 1470 after preliminary incubation in water for release of low molecular weight components

Incubation: 125 mM PPB pH 8.5, 5 mM DTT; 20 h

*100% - released substance after mechanical disintegration of cells with the same concentration

Conclusions

- The release efficiency of total protein and β-galactosidase is similar in the flow and batch system;
- The addition of a reducing agent (DTT) to the incubation medium leads to a significant increase in extraction efficiency;
- DTT has the greatest effect in the first hours of incubation after treatment;
- More significant protein release is observed with higher dilution of the cell suspension in buffer after electroporation;
- Using a two-stage post treatment incubation, the enzyme β-galactosiase can be released in a more purified form;
- During short incubation of electropermeabilized cells in water, a large amount of low molecular weight intracellular components is released;
- Incubation in water (up to 4 h) does not result in inactivation of the enzyme β-galactosidase or decreased release of total protein.

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