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Original Paper

Determination of the efficient enzyme concentration for lytic digestion of vegetative cells but not spores in *Schizosaccharomyces pombe*

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The fact that lytic enzymes such as b-glucuronidase are capable of cell wall lysis, while the integrity of the spore is not affected, is used in the spore viability tests to investigate meiotic processes. Meiosis as a fundamental feature in all living organisms comprises of a complex tightly linked and mutually dependent processes most of which are scientific targets of many research institutions. The fission yeast *Schizosaccharomyces pombe* is a powerful tool for studies on eukaryotic meiosis. Mating of yeast strains of opposite mating types on nitrogen free medium results in spores production. Whereas not all cells undergo meiosis, some cells persist in vegetative stage even in the absence of nitrogen, this leads to generation of a mixed suspension of vegetative cells and spores. Thus, in order to separate spores from vegetative cells obtained mixture was exposed to lytic enzyme action. This treatment kills vegetative cells without affecting spores. To obtain the best and reproducible results of spore recovery and viability, different lytic conditions were analysed. Obtained results show, that use of b-glucuronidase as lytic enzyme for random spore analyses in the fission yeast is dose and time dependent.

Keywords: β-glucuronidase, cell cycle, spore viability, *Schizosaccharomyces pombe*

1 Introduction

The use of cell wall lytic enzymes ranges from biotechnology, medicine, through agriculture to cell biology research. Major applications of these enzymes are related to the extraction of nucleic acids from susceptible bacteria and spheroplasting for cell transformation. Other applications are based on the antimicrobial properties of bacteriolytic enzymes. In addition, transgenic cattle expressing lysostaphin in the milk is characterized by resistance to mastitis caused by streptococcal pathogens and *Staphylococcus aureus* (Donovan et al., 2005). The use of lytic enzymes for the release of recombinant proteins from bacteria has been successful in many cases. For instance, Yang et al. (2000) used a temperature-sensitive lytic system for efficient recovery of recombinant proteins from *Escherichia coli*. Moreover, Žukaite and Biziulevičius (2000) applied lytic enzymes to enhance the production of hyaluronidase by recombinant *Clostridium perfringens* in the course of batch cultivation. Digestion of yeast and fungal cell walls is necessary for many experimental procedures. In the cell biology, cell wall lysis is used for spheroplast production, immunofluorescence, transformation, protein purification, and others. Lytic enzymes such as β-glucuronidase are widely used in yeast genetics to analyze spore viability after meiosis induction. This treatment eliminates vegetative cells and breaks down the ascus wall, while spores remain unaffected. Spores are thereafter used for random spore analysis to investigate spore viability for mutant selection or recombination mapping.

In this study we tested different β-glucuronidase concentrations and different incubation times to estimate the best timing and concentration pattern for disruption of vegetative cells with concomitant spore protection.

2 Material and methods

2.1 Yeast strains, media and growth conditions

Yeast strains:

The genotypes of the yeast strains used in this study are as follows:

- wild type *S. pombe* h+
- wild type *S. pombe* h-

Schizosaccharomyces pombe strains were maintained and grown using standard conditions: complete YE medium with 5 suplements (YES). Meiosis was induced at 25 °C using nitrogen free PMG-N medium.

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Table 1 Identification of remaining vegetative cell counts after β-glucuronidase treatment

Incubation time	% of viable cells		
	4% glucuronidase	2% glucuronidase	1% glucuronidase
2 hours	24.0	25.0	30.6
4 hours	21.0	19.4	23.8
6 hours	20.0	21.7	23.8
8 hours	16.0	13.4	24.1
12 hours	10.0	3.6	15.4
24 hours	0.0	0.0	1.2

2.2 Setting up S. pombe crosses

Freshly growing cells of the opposite mating types on the complete solid YES medium were transferred to liquid YES to achieve cell density of OD_{600} = 0.2. Afterwards, cells were washed 3 times with sterile water. h+ and h- strains were mixed in 30 μL of sterile water and spread as a thin straight line on nitrogen free medium. Cells were incubated at 25 °C for 2–3 days. Mating efficiency was controlled as asci formation using light microscope.

2.3 Random spore analysis

Three days after mating induction presence of asci was checked under the light microscope. Loopful of the cross was inoculated with a 200 μL of YES medium containing either 4, 2, 1 or 0% of β-glucuronidase (*Helix pomatia* juice) and incubated 2, 4, 6, 8,12 or 24 hours at 37 °C. β-glucuronidase is a crude snail gut enzyme that breaks down the ascus wall and kills vegetative cells. The spore number/μL was counted using a Neubauer chamber for cell counting. One hundred spores/plate were plated out on YES Agar medium. The plates were then incubated at 32 °C until colonies were formed.

3 Results and discussion

To obtain mixture of spores and vegetative cells, wild type yeast strains of opposite mating types were crossed and plated on PMG-N sporulation medium to induce nitrogen starvation which in turn leads to meiosis induction (Moreno et al., 1999). Meiosis as specialized cell division ensures genetic variability through meiotic recombination and production of haploid gametes. End products of the yeast meiosis are spores. Random spore analysis is a quick method to generate recombinants or to analyse mating efficiency (Smith, 2009).

After meiosis induction approximately 80% of cells underwent meiosis and formed ascus with four spores.

Three different concentrations of the lytic enzyme β-glucuronidase and six time points were used to investigate the best conditions for elimination of vegetative cells while spores remain unaffected. After each time point, the amount of vegetative cells versus amount of spores were counted and expressed as % of whole cell count (Table 1). The whole cell count represents

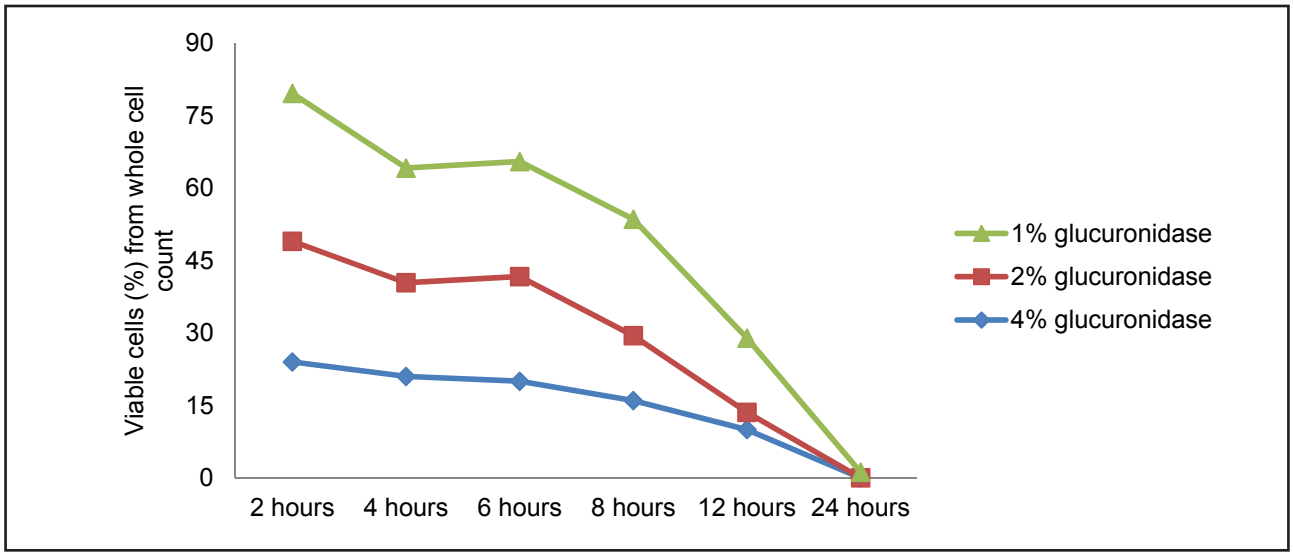

Figure 1 Evaluation of vegetative cell counts from the whole cell count (%)

counts of vegetative cells together with spores and was assessed as 100%.

Treatment of the suspension with β-glucuronidase continuously reduced the amount of vegetative cells in a dose and time dependent manner. Samples with the highest (4%) and middle (2%) concentration of the enzyme were freed from vegetative cells within 24 hours after beginning of the incubation, whereas the lowest enzyme concentration (1%) did not eliminate all vegetative cells (Figure 1). Thus, to reduce the amount of vegetative cells to zero and simultaneously protect spores from enzymatic digestion it is necessary to use at least 2% β-glucuronidase and let it interact at 37 °C or 24 hours. Moreover, spore recovery, after treatment of all β-glucuronidase concentrations was tested and notably, from 100 plated spores, 100 colonies were formed, which points out to 100% spore recovery after enzyme treatment. Other methods of cell wall destruction of vegetative cells have been reported as for instance the use of elevated temperature to achieve enrichment of spores and reduction of vegetative cell counts in suspension. Such method is useful for large screens and use of selective media for spore selection. This method is inexpensive, but does not eliminate all vegetative cell from the suspension. For the complete elimination of vegetative cells another lytic enzymes, such as zymolyase, might be used. Alternatively, it was reported that use of ether preferentially destrois cell walls of vegetative cells rather than spores (Parenti-Castelli et al., 1974; Dawes and Hardy, 1974). Combination of both ether and zymolyase is another possible way of vegeative cell destruction (Bahalul et al., 2010), however this treatment leads to reduction of spore recovery.

4 Conclusions

β-glucuronidase is a suitable lytic enzyme for cell wall digestion of vegetative cells rather then spores.

The best conditions to eliminate vegetative cells while spores remain protected is 2–4% β-glucuronidase treatment at 37 °C for 24 hours. Spore recovery after reported β-glucuronidase treatment is 100%

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