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Quantification of *Saccharomyces cerevisiae* viability using *Bac*Light

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Abstract

Yeast viability can be accurately quantified using *Bac*Light, a kit which so far has been used only for bacterial analysis. Upon staining, viable cells can be differentiated from non-viable ones by either confocal laser scanning microscopy (CLSM), epifluorescence microscopy, or flow cytometry. Using *Saccharomyces cerevisiae* as a model, viabilities quantified by CLSM deviated an average of 1.7% from the actual data, and those determined by flow-cytometry by 1.4%.

Introduction

Quantification of yeast viability is critical for fermentation (Cahill *et al.* 1999), medical examination (Biswas *et al.* 2003), and even in wastewater treatment (Dan *et al.* 2003). In addition, since yeasts often interact with bacteria in nature, the overall viability of yeast and bacteria is also of significance for dental biofilm (Millsap *et al.* 1998) and bioengineering systems such as fermentation reactors (Narvhus *et al.* 2003).

Cell viability is usually defined as the ability of a cell to reproduce and to form colony. It is conventionally measured using the laborious and time-consuming culturing methods. On the other hand, cell viability may also be measured by analyzing the image after staining cells with dyes specific to their metabolic activity (Sami *et al.* 1994, Oh & Matsuoka 2002) or membraneintegrity (Boyd *et al.* 2003). Among all dyes, methylene blue, which reacts with oxidoreductases of the viable cells, is the most widely used (Sami *et al.* 1994).

A commercially available kit, LIVE/DEAD *Bac*Light (Molecular Probes, USA), has recently been developed for the detection of bacterial viability (Lehtine *et al.* 2003, Hope & Wilson 2003). This kit comprises two nucleic acid dyes: SYTO 9 emitting green fluorescence and propodium iodide (PI) emitting red. SYTO 9 stains all cells regardless their viability, whereas PI stains only non-viable cells with damaged membrane integrity. The application of *Bac*Light has so far been limited to bacterial cells, as implied by its name. This present study was conducted to demonstrate that *Bac*Light is also applicable for the determination of yeast cell viability. Images of the stained cells were differentiated using either confocal laser scanning microscopy, epifluorescence microscopy, or flow cytometry. Yeast viabilities quantified using *Bac*Light were also compared to those using the conventional method.

Materials and methods

Sample preparation and plate counting

Four yeast strains were purchased from DSMZ, i.e. *Saccharomyces pastorianus* (DSM6580), *Kluyveromyces marxianus* (DSM4906) and two *Saccharomyces cerevisiae* (DSM70449 & DSM70424), and cultured at 25 °C using modified DSM Liquid Medium 186, which was composed of yeast extract (3 g l^{-1}), peptone (5 g l^{-1}) and glucose (10 g l^{-1}). After 24 h, the medium was centrifuged at 10 000 *g* for 5 min, the yeast pellet was re-suspended in a pH 7.2 phosphate-buffer saline solution (PBS; 0.13 M NaCl in 10 mM

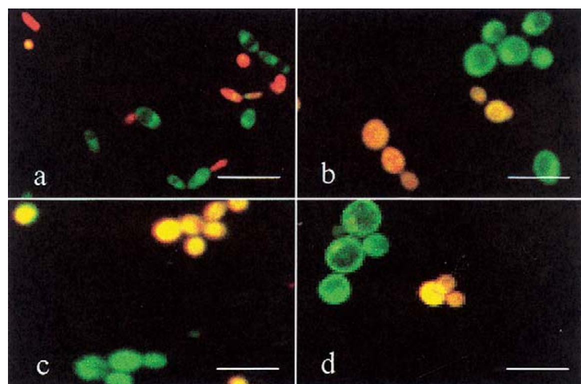

Fig. 1. *Bac*Light-stained images of viable and non-viable yeast cells: (a) *K. marxianus*, (b) *S. pastorianus*, (c) *S. cerevisiae* (DSM70424), and (d) *S. cerevisiae* (DSM70449). Bar = 10 μm.

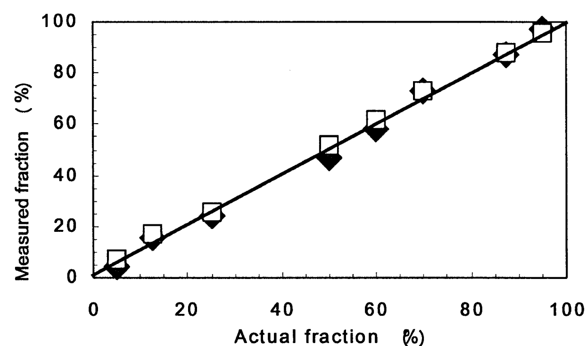

Fig. 2. Comparison between measured fractions of viable *S. cerevisiae* cells using *Bac*Light kit and the actual fractions (♦ and □ were data respectively measured by CLSM and flow cytometry).

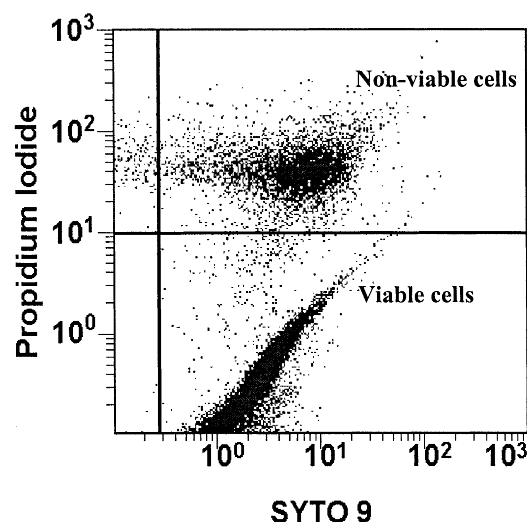

Fig. 4. Flow cytogram of cells of *S. cerevisiae* stained with *Bac*Light (each dot in high PI intensity zone representing a non-viable cell and vice versa).

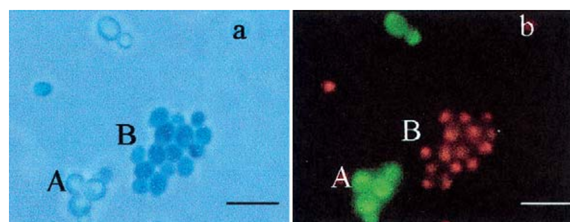

Fig. 5. Images of *S. cerevisiae* cells (A, viable; B, non-viable) in an identical field stained by: (a) Methylene Blue, and (b) *Bac*Light kit. Bar = 10 μm.

Na_2HPO_4), and adjusted the concentration to 10^6 cells ml^{-1} for all subsequent experiments. Non-viable cells were prepared by autoclaving.

Cell staining

The *Bac*Light solution, which contained 100 μM SYTO 9 and 600 μM PI in PBS, was mixed with an equal volume of a yeast solution. Sharp fluorescent images were produced with only 30 s of mixing. The intensities of SYTO 9 and PI were monitored at 480/500 nm and 488-540/617 nm, respectively (Haugland 1996). The methylene blue solution, which contained 0.01% Methylene Blue and 2% (w/v) sodium citrate dihydrate in PBS, was mixed with an equal volume of yeast solution for 10 min. The stained cells in the mixture were quantified under an optical microscope (Nikon E600).

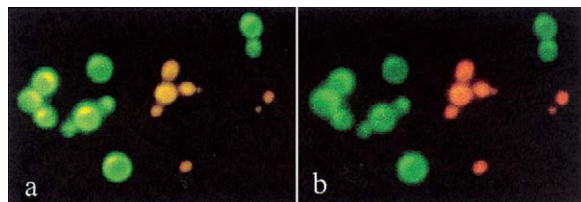

Fig. 3. Images of *S. cerevisiae* cells stained with *Bac*Light under epifluorescence microscope using: (a) fluorescein band-pass filter set (viable cells in green and non-viable cells in yellow), and (b) triple band-pass filter set (viable cells in green and nonviable cells in red). Bar = 10 μm.

Microscopy

A confocal laser scanning microscope (CLSM; model LSM5 Pascal, Zeiss, Jena, Germany) was used. It was equipped with two lasers at 488 and 543 nm, a beam splitter NFT545, two filter sets (BP515-530 and LP560) and a Plan-Apochromat 40× objective (NA0.8, Zeiss). To exclude cross-talk between the detection channels, the two-track mode was applied.

SYTO 9 was excited by a 488 nm laser and the emitting light was collected by the filter BP505-530, while PI was excited by a 543 nm laser and the filter LP560 was used to collect emission light.

The *Bac*Light stained samples were also examined by an epifluorescence microscope (Nikon E600). The filter sets included a fluorescein band-pass filter set (490 nm excitation, 510 nm dichroic and 520 nm emission) and a triple band-pass filter set for 6-diamidino-2-phenylindole (DAPI)–fluorescein–Texas Red. Images were acquired with a Spot Cool CCD digital camera (Marietta, GA).

Flow cytometer

The stained cells were also analyzed using an Epics Altra flow cytometer (Coulter, Miami, FL) equipped with an air-cooled 15 mW Argon ion laser (488 nm) and standard setting of the emission filters. A total of 50 000 cells were counted separately, based on the respective wavelengths, at 250 cells s^{-1} using an EXPO32 software (Applied Cytometry Systems, Sheffield, UK).

Results and discussions

Validity of staining yeast cells using Bac*Light*

The validity of staining yeast cells by *Bac*Light was tested using CLSM, epifluorescence microscopy and flow cytometry. Figure 1 comprises four respective CLSM images of the *Bac*Light-stained yeast cells, i.e. *K. marxianus*, *S. pastorianus*, and two strains of *S. cerevisiae*. Viable cells (in green) and non-viable cells (in red) may be clearly differentiated. The validity of applying *Bac*Light kit for the viability detection of yeast cells was further tested against eight *S. cerevisiae* (DSM70424) samples containing given fractions of viable and non-viable cells. Figure 2 illustrates that measured fractions of viable *S. cerevisiae* cells, using either CLSM or flow cytometry, closely matched with the actual fractions. Viability measurements based on CLSM analysis deviated an average of 1.7% from the actual data, and those based on flow cytometry deviated an average of 1.4%.

Figures 3a and 3b illustrate images of *S. cerevisiae* (DSM70424) cells stained with *Bac*Light using the fluorescein and triple band-pass filter sets, respectively, using epifluorescence microscope. Viable cells (green) can be clearly differentiated from the non-viable cells (yellow). Figure 4 illustrates the fluorescence of viable and non-viable cells were also effectively differentiated using flow cytometry. Dots in the high PI intensity zone (in the order of 10^1 or higher) represent non-viable cells, and the remaining represent viable cells.

Comparison of images stained by Bac*Light and Methylene Blue*

Figure 5 compares the staining images of (a) Methylene Blue and (b) *Bac*Light for an identical field of *S. cerevisiae* (DSM70424). The sample was a 1:1:2 mixture of the yeast suspension, *Bac*Light and Methylene Blue solutions. Viable cells (marked A) were colorless upon Methylene Blue staining (Figure 5a) and corresponded well with those cells emitting green fluorescence upon *Bac*Light staining in Figure 5b. Similarly, non-viable cells in blue (marked B) in Figure 5a correspond well with those cells stained red in Figure 5b. These results demonstrate that viability analysis based on *Bac*Light-stained image was consistent with that using Methylene Blue.

Merit of quantifying yeast cells using Bac*Light*

Yeast cells can be effectively stained using *Bac*Light within 30 s. Viability of yeast cells can be accurately quantified from the stained image. Using *S. cerevisiae* as model yeast, the viabilities measured by CLSM deviated an average of 1.7% from the actual data, and those by flow-cytometry by 1.4%. Furthermore, viable and non-viable cells emitted different fluorescences upon *Bac*Light-staining. Thus, the cell viability may be quantified using flow cytometry which is not applicable for images stained by common dyes such as Methylene Blue.

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