Journal of Radiation Research, Vol. 59, No. 6, 2018, pp. 760–764
doi: 10.1093/jrr/rry070
Advance Access Publication: 25 August 2018

Determining survival fractions of *Saccharomyces cerevisiae* in response to ionizing radiation in liquid culture

Xiaopeng Guo[1,2,†], Miaomiao Zhang[1,2,†], Yue Gao[1,2], Wenjian Li[1] and Dong Lu[1,*]

[1]Institute of Modern Physics, Chinese Academy of Sciences, Lanzhou 730000, China
[2]College of Life Science, University of Chinese Academy of Sciences, Beijing 100049, China
*Corresponding author: Institute of Modern Physics, Chinese Academy of Sciences, No. 509 Nanchang Road, Lanzhou, Gansu 730000, China.
Tel: +86-931-496–9175; Fax: +86-931-496-9201; Email: LD@impcas.ac.cn
†These authors contributed equally to this work and should be considered co-first authors.
(Received 4 March 2018; revised 17 May 2018; editorial decision 23 July 2018)

ABSTRACT

Saccharomyces cerevisiae survival fractions (SFs) in response to X-ray radiation were determined by two new methods: an OD_{600}-based method and a 96-well method. For the OD_{600}-based method, cells were exposed to various X-ray doses and inoculated into fresh medium: a lower biomass accumulated, indicating fewer surviving cells within the investigated dose range (0–100 Gy). For the 96-well method, diluent containing ~0–100 cells was equally divided into 96 droplets and respectively inoculated into 96 wells containing 200 μl of broth: fewer wells without *S. cerevisiae* clones indicated more surviving cells after 48 h of incubation. Corresponding quantitative systems were established. Both methods were sensitive and reliable. The OD_{600}-based method is simple and fast, and the 96-well method simplifies the counting process. SF estimates by the OD_{600}-based method were lower than those by 96-well methods owing to cell cycle arrest. In addition, comparisons of newly proposed and plate-counting methods indicated a higher rate of repair in *S. cerevisiae* in liquid culture than on agar.

Keywords: survival fractions; *Saccharomyces cerevisiae*; X-ray radiation; OD_{600}; 96-well plate

INTRODUCTION

The survival fraction (SF) is routinely used to investigate radiation-induced biological effects, especially to guide mutation breeding by ionizing radiation [1]. In addition to traditional colony-forming unit assays, many valid methods have been developed [2]. However, most of them are effective for mammalian cells, but not for microbes. Therefore, microbial SF determination is still restricted to the plate-counting method in radiobiological studies.

Many damage/repair characteristics are investigated using cells in liquid culture. However, microbial SF determination has usually been performed on agar by the plate-counting method. There are obvious differences in mass transfer area and osmotic pressure between the two; cells incubated in liquid culture possess larger mass transfer area than those on agar; moreover, the decrease in osmotic pressure increases cell survival [3]. Therefore, SFs determined by the conventional plate-counting method may not reflect the survival rate observed when damage and repair are investigated using cells incubated in liquid culture.

Saccharomyces cerevisiae is widely used in radiobiological studies [3, 4]. Here, *S. cerevisiae* SFs in response to X-ray irradiation were determined by two new methods, of which the sensitivity, reliability, characteristics, and advantages are here presented. Comparisons between the methods revealed differences between the ionizing radiation-induced SFs under liquid and solid culture conditions in terms of repair.

MATERIALS AND METHODS

Cell culture and irradiation treatment

Log-phase *S. cerevisiae* strain BY4743 (MATa, budding, amphiploid; American Type Culture Collection, Manassas, VA, USA) cells in

yeast peptone dextrose (YPD) medium were randomly divided into several groups. Samples were X-ray irradiated at 0, 20, 40, 60, 80 or 100 Gy. X-rays were generated using an electronic linear accelerator (Varian-21EX, Palo Alto, CA, USA) at 225.0 kV and 13.25 mA.

OD_{600}-based SF determination

Cells (8×10^6; indicated by N) for each group were inoculated into 100 ml fresh medium in 250-ml flasks and incubated on a mechanical shaker (200 rpm) at 30°C. Additionally, 0.2 N (1.6×10^6), 0.4 N (3.2×10^6), 0.6 N (4.8×10^6), 0.8 N (6.4×10^6) and N (8×10^6) cells from the control group were also inoculated into 100 ml fresh medium. The OD_{600} was then simultaneously determined after 14–16 h incubation. For the control group, the OD_{600} and inoculum size were fitted with an exponential function ($y = A1*\exp(-x/t1) + y0$); the OD_{600} value of each irradiated group was substituted in for y, and the equivalent inoculum size (x) was determined. The SF was expressed as x/N.

SF determination using a 96-well plate

Suspensions from each group were diluted in the same ratios. Next, each 1 ml diluent containing ~60 cells was equally divided into 96 droplets and respectively inoculated into 96 wells with 200-µl YPD broth. After 48 h incubation, a higher number of surviving cells resulted in a lower probability of wells without *S. cerevisiae* clones. This event fitted a typical Poisson distribution, and the cell distribution in each well followed a Poisson function. The total number of viable cells can be computed by the formula: $N = 96*\ln(96/x)$. N indicates total number of viable cells, and x indicates the number of wells without *S. cerevisiae* clones. Furthermore, the plate-counting method was performed as a reference. The same diluents were subjected to plate-counting procedures previously described [1]. Additionally, the cell numbers in the various diluents from the control group (containing 0–100 cells) were determined by the plate-counting and 96-well methods, respectively.

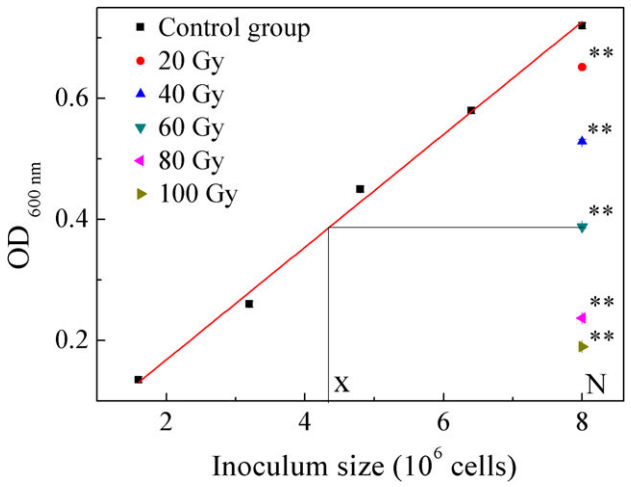

Fig. 1. *S. cerevisiae* SF determination by OD_{600}-based method. The relationships between inoculum size and OD_{600} after certain culture durations can be well described by an exponential curve (as a standard curve). OD_{600} decrease after certain culture durations induced by dose increase is equivalent to that caused by a decrease in inoculum size according to the standard curve. The SFs were determined by x/N. (*$P < 0.05$, **$P < 0.01$ vs the former group).

Cell cycle distribution detection

Detected samples were prepared according to [5], and then immediately analysed using FlowSight (Merck Millipore, Burlington, MA, USA). A cut-off (containing 20% of the cells of the control group with lower PI staining) was set to indicate the cell proportion in the G1 phase.

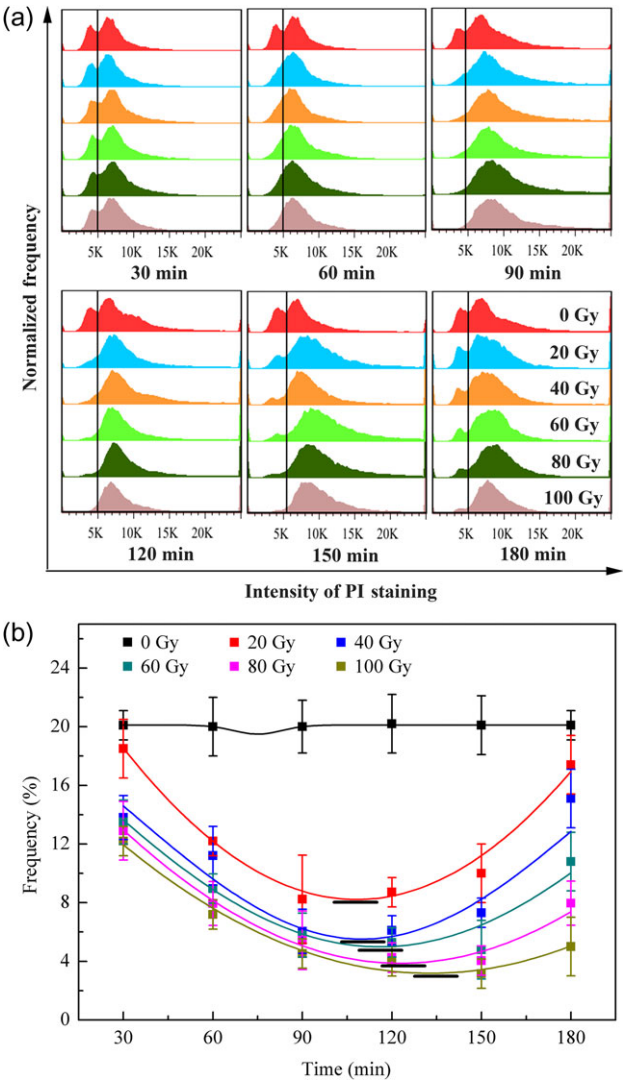

Fig. 2. Dose-dependent cell cycle arrest induced by X-ray irradiation. Cell cycle arrest was intensified and prolonged with increased radiation dose.

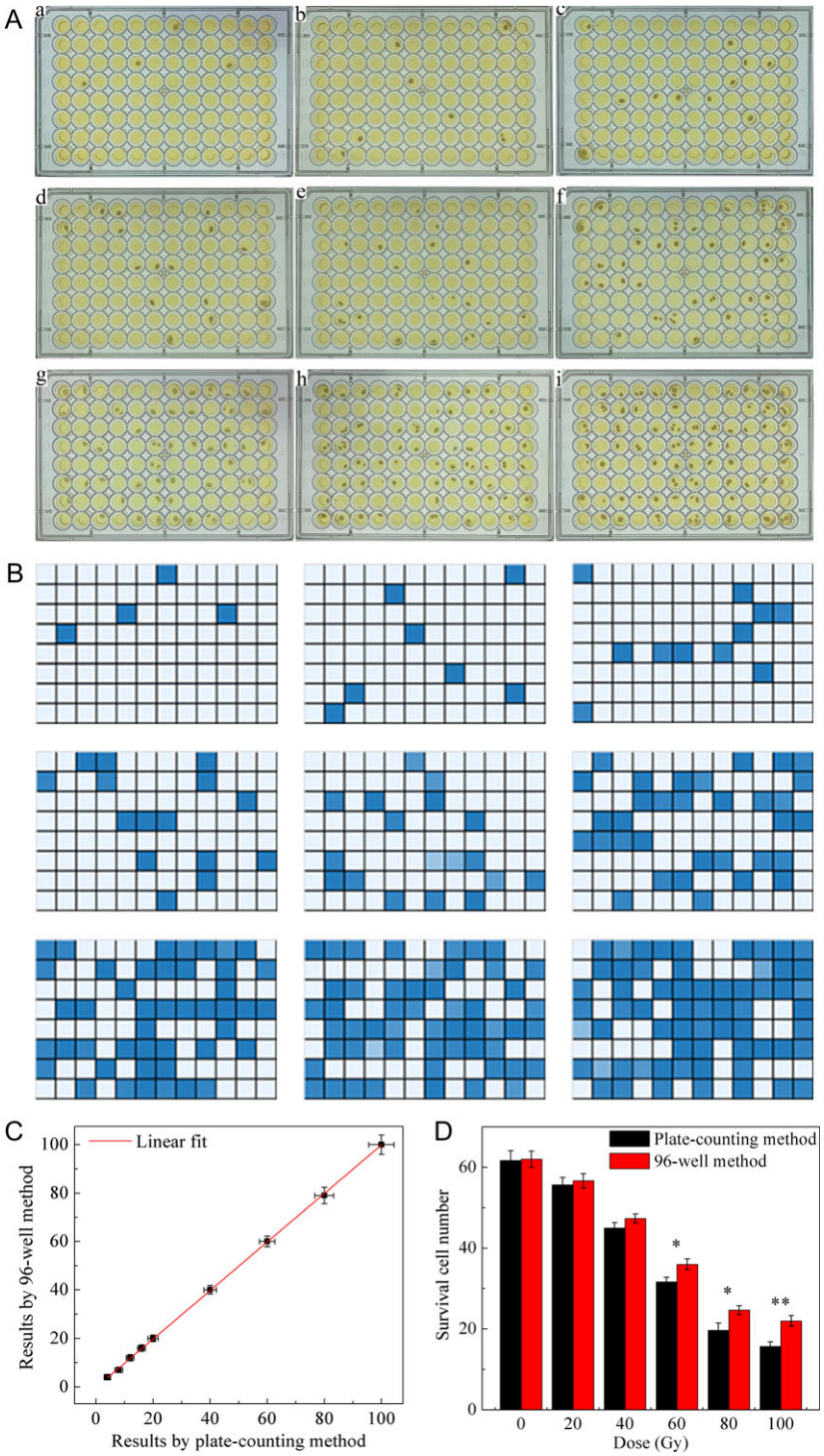

Fig. 3. *S. cerevisiae* SF determination using 96-well method. Each diluent from the control group [containing approximately 4, 8, 12, 16, 20, 40, 60, 80 or 100 cells (A, a–i)] was equally divided into 96 droplets and respectively inoculated into 96 wells. More viable cells were associated with fewer wells without *S. cerevisiae* clones after 48 h of incubation (A, B). The 96-well-method-determined and plate-counting-method-determined numbers of viable cells in different diluents from the control group fitted well with a linear curve (Slope = 1.002, $R^2 > 0.99$) (C), indicating high reliability and sensitivity of the 96-well method. The surviving cell number was determined using the 96-well method and the plate-counting method after varying radiation doses (D).

Statistical analysis

Data are expressed as the mean of at least three independent experiments; bars indicate standard deviations. $P < 0.05$ (one-way variance analysis) was considered statistically significant.

RESULTS AND DISCUSSION

Inverse effects on OD_{600} between dose and inoculum size

The OD_{600} after the same culture duration decreased in response to increasing radiation dose (Fig. 1). Similarly, decrease in inoculum size also corresponded to a reduction in OD_{600}. The relationship between inoculum size and OD_{600} after a certain culture duration could be described satisfactorily by an exponential curve ($R^2 > 0.99$) (Fig. 1), also regarded as the standard curve, to determine the SFs at varying doses. The specific dose that causes a decrease in OD_{600} after a certain culture duration is equivalent to a certain inoculum size according to the standard curve; the SFs can be determined as shown in Fig. 1. The sensitivity and reliability of this OD_{600}-based method are guaranteed by obvious correlations between OD_{600} and radiation dose as well as inoculum size. Meanwhile, the OD_{600}-based method is easy to operate without gradient dilution, spreading on plates, and colony counting.

Impacts of cell cycle arrest

X-ray irradiation–induced cell cycle arrest (Fig. 2a) will delay growth and decrease OD_{600} after a certain culture duration, resulting in a decrease in SF estimates. Furthermore, the statistical data on cell proportions in the G1 phase suggests that an increasing dose caused stronger cell cycle arrest at the same time points and prolonged it (Fig. 2b). Therefore, the decrease in OD_{600} value and SF estimate caused by cell cycle arrest was higher with increasing irradiation dose.

Reliability and sensitivity of the 96-well method

A 96-well method was used to determine *S. cerevisiae* SFs in liquid culture, which ensured that cells were incubated in liquid culture and allowed the counting of viable cells (Fig. 3A, B). As shown in Fig. 3C, the different numbers of viable cells from the control group were counted by the 96-well method based on the Poisson function, which was linearly dependent on the plate-counting method–determined value with the investigated range of 0–100 (Slope = 1.002, $R^2 > 0.99$). Thus, SF determination in liquid culture using a 96-well plate was sensitive and reliable. Based on this, the surviving cell numbers for different groups were respectively determined by the 96-well method in liquid culture and by the plate-counting method in solid culture (Fig. 3D).

Comparisons between methods

The essential distinctions between the 96-well method and the plate-counting method are the counting process and the repair conditions. For the plate-counting method, single colonies were randomly dotted on the plate. There may be several colonies at a location, and the accurate reading on colonies number at this location should be carefully identified as 2, 3, or so on. When cells were clustered, seriously damaged cells were at a competitive disadvantage for nutrition and inclined to form smaller colonies relative to those with slight damage, which made counting more difficult. For the 96-well method, the reading was just as 'Yes' or 'No' for each well, and the cells in different wells were isolated from one another. Thus, the 96-well method simplifies the counting process. Additionally, the SFs were found to be higher by the 96-well method than by the plate-counting method (Fig. 4), indicating that the SFs were higher in liquid culture than on agar. The differences were significant in the 60, 80 and 100 Gy groups, in which more sublethally injured cells existed. These data suggest that liquid culture was better than solid culture for completing the repair of irradiated cells, thereby increasing the SF estimates.

Cell cycle arrest barely influenced the count results or the corresponding SFs estimates in the 96-well and plate-counting methods. However, it reduced the SF estimates by the OD_{600}-based method. Meanwhile, the OD_{600}-based method–determined SF estimates were increased by the greater ability to repair in liquid culture than on agar. Radiation-induced dead or reproductively dead cells from inoculums may also have slightly increased the OD_{600}-based method–determined SF estimates by contributing to the OD_{600}. Here, we specially selected a very small inoculum size and a large culture system. Therefore, the increase in OD_{600} caused by the dead or reproductively dead cells from inoculums was negligible. The survival curve determined by the OD_{600}-based method was below that determined by the 96-well method (Fig. 4). However, there were no significant differences between the OD_{600}-based method–determined and plate-counting method–determined SFs ($P > 0.05$) (Fig. 4), suggesting a balance among the factors that would lead to an increase or decrease in SF estimates when comparing the OD_{600}-based method with the plate-counting method.

Overall, the OD_{600}-based method and the 96-well method are promising ways for determining SFs of *S. cerevisiae* or other single-

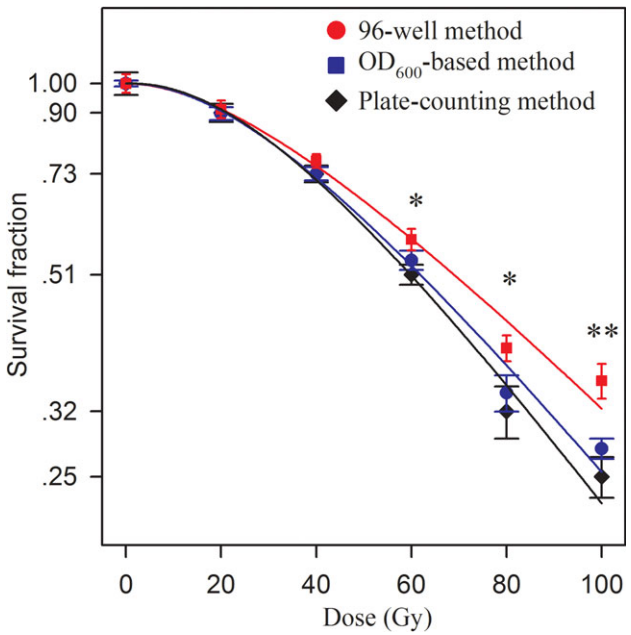

Fig. 4. Survival curves determined by plate-counting, OD_{600}-based and 96-well methods, respectively. (*$P < 0.05$, **$P < 0.01$, SFs by 96-well method vs plate-counting method).

celled microbes in response to X-ray or other ionizing radiations, and have distinctive advantages. Determining the SFs in liquid culture is the unique property of the newly proposed methods; the comparisons between the methods revealing a greater ability of *S. cerevisiae* to complete repair in liquid culture than on agar. This study may further other relevant radiobiological studies.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

FUNDING

This work was supported by Chinese Academy of Sciences Key Deployment Project (No. KFZD-SW-109), Joint project of Chinese Academy of Sciences and Industrial Technology Research Institute (CAS-ITRI 201801) and the National Natural Science Fund of China (No. 11575259).

REFERENCES

1. Toyoshima Y, Takahashi A, Tanaka H et al. Lethal and mutagenic effects of ion beams and γ-rays in *Aspergillus oryzae*. *Mutat Res* 2012;740:43–9.
2. Lu Y-Y, Chen T-S, Qu J-L et al. Dihydroartemisinin (DHA) induces caspase-3-dependent apoptosis in human lung adenocarcinoma ASTC-a-1 cells. *J Biomed Sci* 2009;16:16.
3. Wang M-S, Zeng X-A, Sun D-W et al. Quantitative analysis of sublethally injured *Saccharomyces cerevisiae* cells induced by pulsed electric fields. *LWT-Food Sci Technol* 2015;60:672–7.
4. Moscariello M, Sutherland B. *Saccharomyces cerevisiae*–based system for studying clustered DNA damages. *Radiat Environ Biophys* 2010;49:447–56.
5. Krishan A. Rapid flow cytofluorometric analysis of mammalian cell cycle by propidium iodide staining. *J Cell Biol* 1975;66:188–93.