Survival rate of yeast cells in different storage media

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Yeast fermentation has played a vital role in food production throughout the millennia of human history. Notable examples of its applications include alcoholic beverages, such as wine and beer, as well as breads found globally.[1] Besides its extensive industrial applications, yeast is an extensively used model organism in studies on ageing,[2] population genetics and other topics.[3] It is a highly common target organism in irradiation experiments and ensuing mutation breeding at the Beam Mutagenesis Group. It typically takes 3–5 days from the preparation of the target samples for irradiation until they arrive at the partner institution for post-experiment examination. During this time, including that for irradiation itself, yeast cells are suspended in a liquid medium, for which phosphate-buffered saline (PBS) is commonly used. However, its effect on cell viability, including the possibility of using alternative media, has not been fully elucidated. Therefore, we tested the survival of yeast cells in three different storage media over time, simulating the time frame of common irradiation experiments.

A wild-type bakers' yeast, *Saccharomyces cerevisiae* BY4743, was chosen as the model. Three storage media including ultrapure water (Milli-Q water), PBS (8.1 mM disodium hydrogen phosphate, 1.47 mM potassium dihydrogen phosphate, 2.68 mM potassium chloride, 137 mM sodium chloride) and 10 mM magnesium sulfate ($MgSO_4$) were tested.

First, a well-isolated colony was inoculated into a test tube filled with 5 mL YPD broth (10 g yeast extract, 20 g meat peptone and 20 g D-glucose, per litre). A portion of this pre-culture was transferred to 20 mL YPD in a 125-mL baffled flask. The culture was incubated at 30°C, shaking at a rate of 225 rpm.

Second, *S. cerevisiae* cells were harvested by centrifugation of 12 mL of the above culture and washed thrice with one of the three tested storage media. The cell concentration was adjusted to $OD_{600} \approx 1.0$ (equivalent to $\approx 10^7$ cells/mL) to replicate a typical irradiation target. After a given time at 4°C, 100 μL of the solution was diluted with 10 mM $MgSO_4$ and 30 μL of the diluted solution was spread on five YPD plates.

Finally, after approximately two days of incubation at 30°C, we recorded the number of colonies in each plate. The survival rate was calculated based on the mean colony count of three plates, eliminating the two plates with maximum and minimum colony counts, respectively.

We repeated the above experiment thrice. The most representative result from the three trials is shown here.

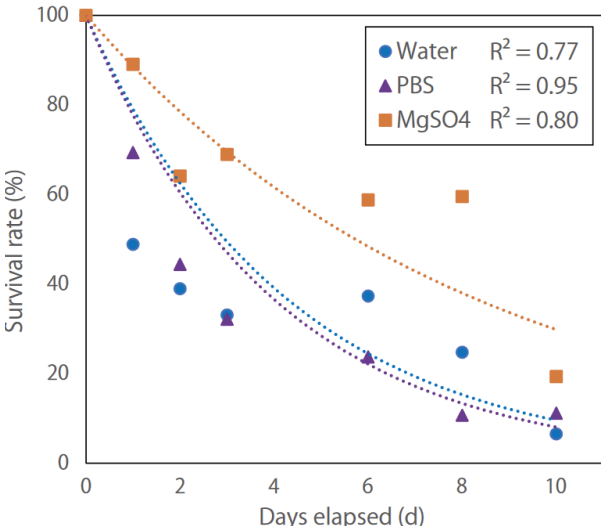

Fig. 1. Survival rate of BY4743 expressed relative to Day 0 control value set as 100%.

Figure 1 shows the survival rate of *S. cerevisiae* over ten days, in which the dotted lines represent the exponential regression models applied to the data of the three tested storage media.

For all of them, the coefficients of determination, R^2, between the days elapsed and the survival rate, are greater than 0.7. Storage in PBS and water resulted in similar trends wherein approximately 40% cells die after only two days. In contrast, 10 mM $MgSO_4$ allows for a relatively higher rate at which approximately a half of the viable cells survives for six days.

The above results show that the viability of the yeast cells significantly differs between the tested storage media. Despite the prominent use of PBS as a storage medium, our results indicate that the survival rate of the yeast cells therein is similar to that in water and hence, PBS is a non-ideal medium for long-term storage. In contrast, based on the higher survival rate, 10 mM $MgSO_4$ is more recommendable for its use as a yeast storage medium.

References

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