Effect of proline on yeast cells' recovery from heat shock

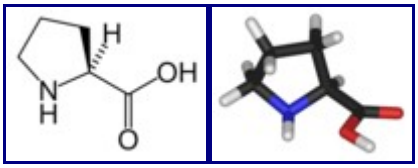
Abhishek Dasgupta
Indian Institute of Science Education and Research, Kolkata

Abstract This experiment aims to observe the effect of high temperatures on the growth of yeast and to find if proline helps in the recovery of yeast cells from heat shock at various temperatures.

Keywords yeast, heat shock, proline, recovery

Introduction

Yeast (*Saccharomyces cerevisiae*), a very common eukaryote suffers from heat shock when the temperatures are raised to high levels. Some yeast manage to sporulate, others die. When temperature rises beyond the optimum temperature, many proteins stop being synthesised and new proteins are mare synthesised to protect the cell in the altered environment. In this experiment we add proline to the yeast cells before heating to observe the effect (if any) it may have on the growth of yeast cells. Proline is a very well known amino acid. The distinctive cyclic structure of proline's side chain locks its φ backbone dihedral angle at approximately -75°, giving proline an exceptional conformational rigidity compared to other amino acids. Hence, proline loses less conformational entropy upon folding, which may account for its higher prevalence in the proteins of thermophilic organisms. So proline's folding is much affected by heat.



In recent years, research has found out that proline has a protective action against many forms of environmental fluctuations for many organisms including some kind of fungi. We will investigate any possible protective nature of proline in this experiment.

Experiment

We took a 0.088 OD yeast culture grown in YPD (yeast extract, peptone and D-glucose) medium. Into each of 6 sterilised Eppendorf tubes, 1mL of the above culture is added, the mouth is wrapped with parafilm, to prevent evaporation and opening up and they are placed under different conditions- 30 ℃

(this is the control, for 30 mins), 50 ℃ (for 5 min), 50 ℃ (for 15 min), 50 ℃ (for 30 min), 60 ℃ (for 30 mins). Amount of proline added 2 millimolar (25 µL).

After treatment, the Eppendorf tubes are directly plunged into an ice-bath to stop further growth of yeast. Next we make an estimate of the recovery of yeast from heat shock at room temperature with or without proline by employing two methods:

(i) *plotting a growth curve (optical density versus time)*: For this, following heat treatment, 0.1mL of culture from each of the 12 eppendorf tubes is added to 12 different flasks, with 20mL of YPD medium each.

(ii) *viability counts*: The rest of the culture from each of the twelve eppendorf tubes is diluted serially and we spread plate for viability counts at different dilution factors.

Results and Discussion

Table 1 viability counts.

temperature (℃)	time (min)	dilution factor	amount (µL)	count
50	30			17
50	30			19
60	30			13
50	5			89
50	15			3
60	30			12
50	5			113
30	30			652
30	30			103
50	15			484
50	5			18

Table 2 spectrophotometer reading of the OD of the differently heat-treated cultures after 15 hours.

proline?	temperature (℃)	time (minutes)	optical density
no	30	-	0.128
no	50	5	0.001
no	50	15	0.003
no	50	30	0.003
no	60	30	0.000
yes	30	-	1.076

yes	50	5	0.233
yes	50	15	0.150
yes	50	30	0.108
yes	60	30	0.169

The graph of optical density of the culture (which is directly proportional to growth of the yeast) is plotted against temperature and time (for constant temperature 50 ℃). The graph clearly shows a decrease in growth as temperature is increased. At **60 °C**, which is above the lethal dose for yeast, all yeast is exterminated, and the optical density relative to the medium drops to zero.

Acknowledgements

I wish to thank our helpful biology instructors, Dr. J. Bhattacharya, Dr, Tapas Sengupta and Dr. N.C. Mandal who inspired and guided us in the successful execution of this project.

References

1. Cell Biology *Gerald Karp*
2. GENES VIII, *Lewin*