# Wild type versus mutant yeast cells

ASSIGN BUSTER

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The yeast used in this experiment is S. cerevisiae. Verprolin protein which encoded by VRP1 is required in cell growth and cytoskeletal organization. However, when VRP1 is mutated, it is sensitive to high temperature and normal function verprolin protein cannot be produced properly and actin polymerization of the cells is inhibited. Besides, it also proliferates in a slower rate in room temperature. This experiment proved that mutated yeast cells can be rescued by transforming normal VRP1 gene into the yeast cells. The rescued yeast cells can then function like wild type yeast cells and can grow under higher temperature.

## Introduction

Wild type yeast cells contain VRP1 gene that encodes Verprolin protein. VRP1 protein is the yeast (S. cerevisiae) ortholog of human Wiskott-Aldrich syndrome protein (WASP)-interacting protein (WIP). WASPs are one of the proteins that regulate actin polymerization. They concentrate to the cortical actin cytoskeleton and are required for its polarization to sites of growth and are also essential for endocytosis. In yeast, the Arp2 â), 3 complex localizes

to cortical patches that partially colocalize with cortical actin patches like VRP1 protein, Las17p, and type I myosins. Las17p and type I myosins promote the accumulation of actin monomers into actin filaments by binding and activating the Arp2 â), 3 complex. Therefore, destruction of VRP1 gene inhibits the growth of cells because VRP1P is the key regulator of cortical actin polymerization. However, VRP1 protein becomes essential for growth at higher temperature which is raised from 28°C to 37°C (Thanabalu et al. 2007). Besides, the mVRP1 yeast cells can be rescued by the transform the normal functioning VRP1 into the mVRP1 yeast cells. The cells now should perform the same way as wild type yeast cells.

## Aims

1. To investigate the differences between wild-type yeast and mutant yeast (mVRP1 gene) on their morphology and proliferation in cell culture plate at different temperature.

2. To investigate if the normal VRP1 gene from DNA plasmid is transformed into the mutant yeast and replaced the mutated VRP1 gene by observing their colonies in the cell culture plate.

## Methods

To observe the morphology of wild type and mutant yeast, Wild type yeast cells and mutant yeast cells are pipetted onto two different microscope slides and covered them with coverslip. The size of 20 cells for each slide was observed and measured under a microscope with x40 of magnificent.

To investigate the proliferation of yeast cells, two YPD plates (37°C and RT) were prepared and each of the plate was divided into two parts (WT and

mVRP1). Streaked an aliquot of WT yeast cells and mutant yeast on the correct parts for both plates. The plates were then left incubated under the temperature required. The colonies on the plates were examined after one week.

To allow the PCR reaction for wild type and mutant yeast cells, plasmid DNA was isolated from the cell cultures. Tubes for “ mVRP1”, “ WT” and “ negative control” were prepared. mVPR1 and WT tubes contained master mix and appropriate DNA template while control tube contained master mix and sterile water. They are then transferred to thermal cycle to allow PCR reaction to occur. After one week, loading dye is added to the PCR reaction products. 100bp DNA ladder and the two solutions were loaded and ran in agarose gel. The bands appeared were observed.

To study the transformation of mVRP1 tube and wild type yeast cells, transformation mix solution was added to 2 microcentrifuge tubes which one contained denatured salmon sperm and plasmid DNA (plasmid) and another one contained denatures salmon sperm and sterile water (control). Purified mVRP1 yeast cells were then pipetted into each of the tubes above. After incubation, each of the solutions were then spread onto two plates (37°C and RT) and incubated under the temperature required. The plates were then observed.

## Results

After the microscope slides were observed under microscope, the population morphology was found to be different between the WT and mVRP1 cells. WT cells are homogenous in their size, whereas mVRP1 cells are heterogeneous

in their size. Besides, WT cells grow in clumps and mVRP1 cells grow singly. The size of 20 cells is WT and mVRP1 cells were measured and found that the average size for WT cells is 4. 8µm and for mVPR1 cells is 3. 9µm.

Other than that, both mVRP1 and WT cells grew in the plates when incubated under room temperature. mVRP1 cells proliferated slower compared to WT cells since less colonies were form in the mVRP1 region. However, mVRP1 cells do not form colonies when incubated under 37°C but WT cells form colonies which is same as the colonies formed by WT cells under room temperature.

Besides, the bands formed in agarose gel were observed. The plasmids were run from cathode of the gel to anode of the gel. Each of the plasmid presented one band on the gel. WT plasmid has a band which runs slower than mVRP1 plasmid. Thus the molecular weight of WT plasmid is greater than mVRP1 plasmid. The molecular weight of mVRP1 and WT plasmids were measured based on the 100bp DNA ladder.

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Figure 1: This gel image shows the difference size of the plasmid from wild type yeast cell and mVRP1 yeast cell. The first band formed is belong to wild type plasmid while the second one is mutant plasmid.

Colonies were found on the control plate that containing no plasmid, mVRP1 cells and was incubated under room temperature. However, colony was not found in the control plate when it was incubated under 37°C. Colonies were found on the experimental plate which contained VRP1 plasmid, mVRP1 cells

and was incubated under room temperature. This shows that the VRP1 plasmid is transformed into the mVRP1 yeast cells. Colonies were also found on the experimental plate that was incubated under 37°C but it was less than the colonies in experimental plate under room temperature. mVRP1 cells proliferated slower than WT cells at room temperature and high temperature inhibited the proliferation of mVRP1 while cause no effect on WT cells.

## Discussion

WT cell has a bigger average cell size and faster rate of proliferation than mVRP1 cell. This is because wild type yeast cells have normal functioning VRP1 gene and can produce VRP1P. The interaction between short sequences in VRP1P with proteins such as actin, type I myosins, and Las17p may be required individually to keep the cells grow and move (Thanabalu et al. 2007).

No colonies in the plate with mVRP1 yeast cells which was incubated under 37°C indicates that they do not grow in high temperature whereas WT yeast cells can move and grow in normal condition. This is because mutated VRP1 gene is temperature sensitive therefore its function is inhibited under higher temperature. When mVRP1 yeast cells are incubated under elevated temperature, it promotes protein accumulation. Furthermore, proteins which interact with VRP1 protein such as actin and Las17p are proteins that will have deleterious effects in cell growth and actin polymerization especially under higher temperature (Thanabalu et al. 2007). As a result, the mVRP1 grow in clump.

The length of mVRP1 plasmid is shorter than the wild type plasmid. This is because the mutation of the VRP1 gene may cause some deleterious of sequences within the plasmid. Therefore, the mvRP1 plasmid is shorter than the wild type plasmid.

There are lesser colonies on the experimental plate under 37°C because not all of the VRP1 plasmids are transformed into the mVRP1 yeast cell during the incubation. Thus, the mutant cells that without the VRP1 plasmid transformed will die off and thus, forming fewer colonies.