Bakers’ yeast, Saccharomyces cerevisiae, is an eukaryotic microorganism that is used in basic biological research and is favored by most (2). For this reason, many people have looked into yeast and how it can be used as a model organism for a mammalian heart cell (1). Calcium is the main signaling molecule in all eukaryotic cells and it plays an important role in things like cell division in yeast (5). Iron was placed into the cell using a luminometer assay at different concentrations in order to see the affect it had on the calcium homeostasis in the yeast cell. Calcium is transported into the vacuole via mutants Vcx1p, Pmc1p, and Pmr1p. The wild type yeast is a yeast cell with Vcx1p, Pmc1p, and Pmr1p. The wild type yeast is a yeast cell with calcium transport pathways being triggered from stress, further research needs to be conducted. Next steps will need to include the use of mutants that are specifically for ferrous sulfate ion channels. Such as, Ftr1p, Fet3p, Fet1p2p, and Fet4p.

Introduction

Bakers’ yeast, Saccharomyces cerevisiae, is an eukaryotic microorganism that is used in basic biological research and is favored by most (2). For this reason, many people have looked into yeast and how it can be used as a model organism for a mammalian heart cell (1). Calcium is the main signaling molecule in all eukaryotic cells and it plays an important role in things like cell division in yeast (5). Iron was placed into the cell using a luminometer assay at different concentrations in order to see the affect it had on the calcium homeostasis in the yeast cell. Calcium is transported into the vacuole via mutants Vcx1p, Pmc1p, and Pmr1p. The wild type yeast is a yeast cell with calcium transport pathways being triggered from stress, further research needs to be conducted. Next steps will need to include the use of mutants that are specifically for ferrous sulfate ion channels. Such as, Ftr1p, Fet3p, Fet1p2p, and Fet4p.

Hypothesis

High concentrations of iron can weaken or disrupt yeast cells, causing an influx of calcium into the vacuole of the yeast cell.

Why it’s important?

Yeast has the same components, structure and function along with actions like mammalian cells. Synthesis, analysis, and regulation as seen in mathematical models (1). Looking for how metals affect the heart, iron is an element that is found in many households ranging from the drinking water to the dust around houses (3).

Experiment

Yeast needed to be prepared for the transformation, so it was grown in an overnight culture, containingYPD glucose and three to five colonies of cells in the incubator shaker (Figure 2) that was taken from a prepared plate of cells (Figure 7). Transformation of the normal yeast cells and three mutant cells using Frozen-EZ Yeast Transformation II. This was in order to insert the plasmid that contained the gene that encoded for aquaporin protein.

The yeast then needed to be prepared for the luminometer assay, the strains that were transformed were then grown overnight in culture, containing YNB glucose, yeast cells, and the appropriate nutritional supplements. A back dilution was necessary in order to get cells to be at the desired concentration of 0.4 (A600nm) which was measured in spectrophotometer (Figure 8), and the colonies were put into the incubator shaker (Figure 4) for two hours. A coelenterazine treatment was then done to activate aquaporin in each of the strain colonies with centrifuging the colonies in a centrifuge(Figure 5) in tubes and then in a microcentrifuge (Figure 6). The microtube tubes were then covered with aluminum to keep light out.

For the luminometer assay, the luminometer (Figure 9) was primed with iron which will pulse through the cells at the 30 second point of the calcium kinetics assay. The samples were put into the instrument in 200 microliter samples in each of the strains of cells and measured over a 3 minute 40 second time. Additionally, a 56 second Lmx-assay was carried out for each of the strains as well with a detergent solution put in before the rest was run. Using the highest Lmax value and the values of the calcium kinetics assays graphs were created using formulas that give a total cytosolic calcium ion concentration. The Lmax is used to normalize the data gathered. Once the data was collected these values were put into the y-axis and then the time was put into the x-axis over the time.

Results

Cytosolic Calcium Levels

Figure 10. Cytosolic calcium levels, in the logarithmic scale, in Water (blue), 1 Molar (red), and 0.25 Molar (Green) concentrations of iron added to the wild type yeast cell in minutes and seconds.

Figure 11. Cytosolic calcium levels, in the logarithmic scale, in mutant strain lacking VCX1 in 1 Molar (red) and 0.25 Molar (Green) concentrations of iron added to the wild type yeast cell in minutes and seconds.

Figure 12. Cytosolic calcium levels, in the logarithmic scale, in mutant strain Pmc1Δ in 1 Molar (red) and 0.25 Molar (Green) concentrations of iron added to the wild type yeast cell in minutes and seconds.

Conclusions

The yeast proteins tested posses the same functional similarities as cardiac myocytes. Vcx1p (yeast) is compared to the cardiac protein NCX1(cardiac). Both proteins are exchangers that mediate the removal of cytoplasmic Ca2+. Pmc1p (yeast) and SERCA2 (cardiac) proteins mediate the sequestration of cytoplasmic Ca2+ into the ER/RS and are regulated by cytosolic Ca2+. Yeast and mammalian cardiac myocytes have calcium transport proteins that can impact homeostasis.

What was determined from this research is that the higher the concentrations of ferrous sulfate showed there is an influx of calcium that enters the yeast vacuole. In order to fully comprehend which transport pathways are being triggered from stress, further research needs to be conducted. Next steps will need to include the use of mutants that are specifically for ferrous sulfate ion channels. Such as, Ftr1p, Fet3p, Fet1p2p, and Fet4p.

References


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