Experiment 1: Isolation and Characterization of Macromolecules

Flow Chart and Post-Lab

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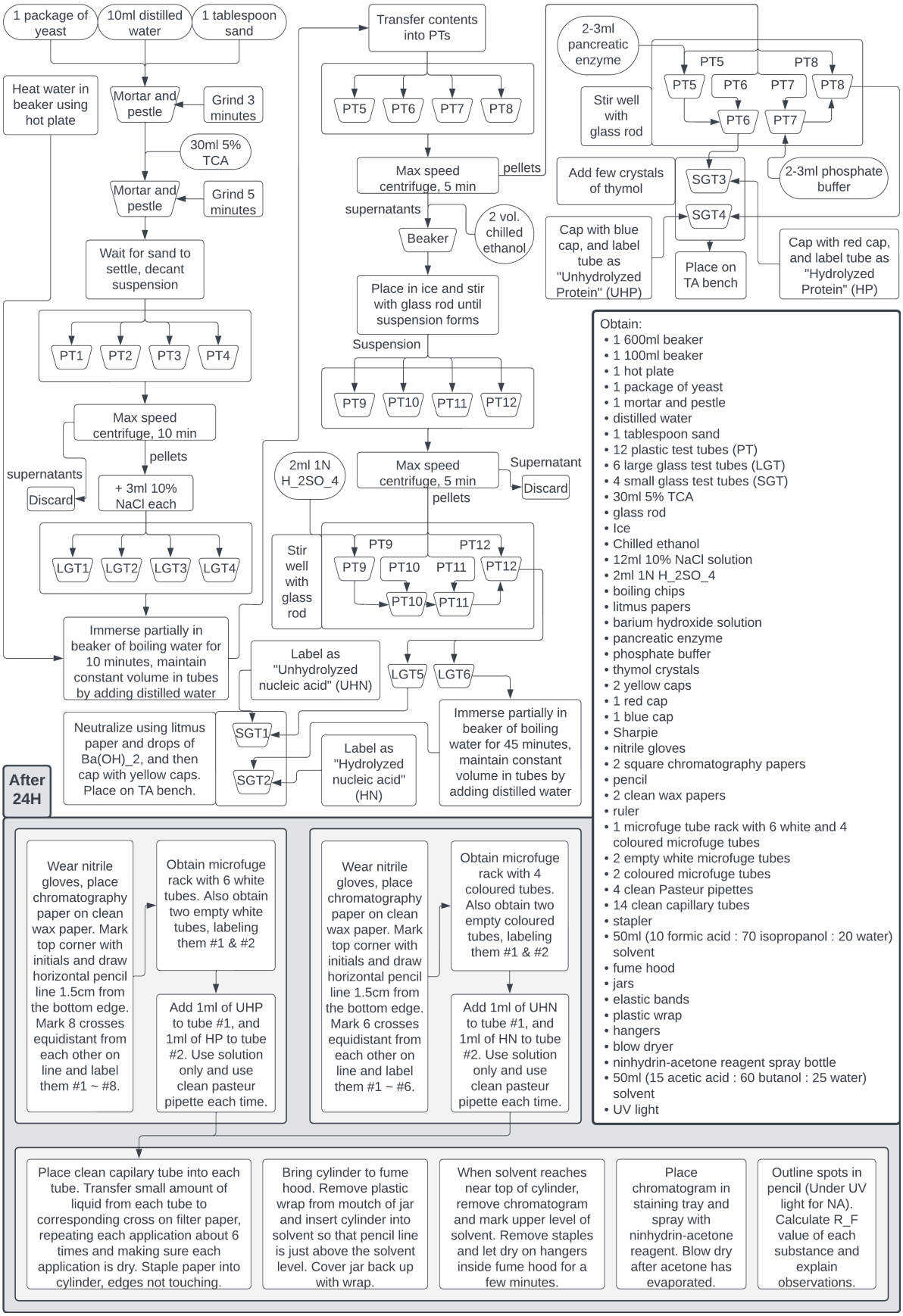
Flow Chart

Steps were given in (Online Lab Manual, 2023).



Summary

This experiment's objective was to isolate and characterize macromolecules from yeast cells, a model organism used to study human physiology, development and diseases. Important techniques used during the lab include centrifugation; used to separate pellets from supernatants in solutions, and chromatography; used to distinguish individual compounds from the protein and nucleic acid mixture samples. In the chromatography results for hydrolyzed protein, distinct smudges indicated presence of lysine, alanine, and aspartic acid in the sample. In the results for hydrolyzed nucleic acid, adenine was detected; however, no other matches to other base subunits were found, indicating unsuccessful conduction of the procedures. Overall, this lab demonstrated the techniques to find the composition of macromolecules in yeast cells, with some unexpected results that emphasized the importance of careful lab techniques for accuracy.

Results

Sample Calculation

$$R_{f_{\text{alanine}}} = \frac{\text{Distance (from origin) travelled by substance (cm)}}{\text{Distance (from origin) travelled by solvent (cm)}} = \frac{9.1\text{cm}}{11.9\text{cm}} = 0.76$$

Protein Chromatogram

$$\begin{aligned}
R_{f_{\text{unhydrolyzed protein (dark purple smudge)}}} &= 0.57 \\
R_{f_{\text{unhydrolyzed protein (pink smudge)}}} &= 0.71 \\
R_{f_{\text{hydrolyzed protein (dark purple smudge)}}} &= 0.57 \\
R_{f_{\text{hydrolyzed protein (light purple smudge)}}} &= 0.76 \\
R_{f_{\text{hydrolyzed protein (very light purple smudge)}}} &= 0.94 \\
R_{f_{\text{alanine}}} &= 0.76 \\
R_{f_{\text{histidine}}} &= 0.46 \\
R_{f_{\text{aspartic acid}}} &= 0.56 \\
R_{f_{\text{lysine}}} &= 0.57 \\
R_{f_{\text{methionine}}} &= 0.88 \\
R_{f_{\text{unknown amino acid}}} &= 0.57
\end{aligned}$$

Protein Chromatogram — Explanation

In the chromatogram for hydrolyzed protein, there are three to four distinguishable smudges from the ninhydrin. The dark purple smudge in the center has an RF value of 0.57, which matches that of lysine. The purple smudge above it has an RF value that matches one of alanine. Hints of pink can be seen hidden by the dark purple smudge; as ninhydrin produces different types of purple depending on the chemical nature of the amino acid it reacts with (Perrett and Nayuni, 2014), the hidden pink smudge can be matched to aspartic acid in shade and in RF value. The faint purple smudge at the very top may represent methionine which its RF value is most similar to, as methionine does exist in small amounts in yeast protein (Podpora et al., 2016). The unknown amino acid is most likely lysine as it has an RF value of 0.57 which matches the lysine sample given.

It is unexpected that the unhydrolyzed protein also produced visible smudges, as no amino acids should have been present to react with ninhydrin. This may have been caused by contamination during the lab process, introducing amino acids into the sample and creating visible smudges.

Nucleic Acid Chromatogram

$$
\begin{aligned}
R_{f_{\text{unhydrolyzed nucleic acid}}} &= \text{N/A} \\
R_{f_{\text{hydrolyzed nucleic acid (furthest smudge)}}} &= 0.65 \\
R_{f_{\text{hydrolyzed nucleic acid (middle smudge)}}} &= 0.43 \\
R_{f_{\text{hydrolyzed nucleic acid (closest smudge)}}} &= 0.23 \\
R_{f_{\text{adenine}}} &= 0.65 \\
R_{f_{\text{cytosine}}} &= 0.51 \\
R_{f_{\text{uracil}}} &= 0.57 \\
R_{f_{\text{adenine-cytosine-uracil (furthest smudge)}}} &= 0.64 \\
R_{f_{\text{adenine-cytosine-uracil (closest smudge)}}} &= 0.47
\end{aligned}
$$

Nucleic Acid Chromatogram — Explanation

In the chromatogram for hydrolyzed nucleic acid, there are three spots marked after UV light visualization. The spot farthest from the initial line matches the RF value of adenine most. The other two spots of the hydrolyzed nucleic acid does not seem to match the RF values of the given samples. This is most likely from contamination during the lab process from excess supernatents, or salts.

The unhydrolyzed nucleic acid produced no visible spots as expected; the adenine-cytosine-uracil sample seem to match the individual base samples, with uracil not being marked due to close proximity with adenine.

Discussion Questions

Why is it important to maintain a constant volume of water in your water bath (when boiling nucleic acid fractions)?

If the water bath is not maintained at constant volume, it will eventually evaporate to the point where the nucleic acid fractions are no longer submurged in the water bath. This can cause uneven heating of the samples which would lead to inaccurate results.

Why are test tubes with red caps incubated at 37 °C? Why are the test tubes kept in the refrigerator until they are to be used for chromatography?

The pancreatic enzymes added to the test tubes with red caps are incubated at 37 °C to simulate body tempurature which the enzymes are active in. During incubation, the enzymes hydrolyzes the protein molecules into amino acid subunits. Once the tubes are finished incubating for 24 hours, they are refrigerated until chromatography to prevent contamination and keep the hydrolysed contents of the tube stable.

If all of the isolation and fractionation experiments were 100% successful, what substances or molecules would you expect to be present in:

(a) **The tube labelled "hydrolyzed protein"**

Amino acids, such as lysine, leucine, aspartic acid, glutamic acid, and isoleucine which are major components of yeast protein (Abdel-Hafez et al., 1977).

(b) **The tube labelled "unhydrolyzed protein"**

As no hydrolysis of protein took place, there would be no amino acids in the tube, only whole protein molecules.

(c) **The tube labelled "hydrolyzed nucleic acids"**

As the amount of RNA is much greater than the amount of DNA in yeast cells (Online Lab Manual, 2023), the hydrolyzed nuclic acid will contain RNA base subunits uracil, cytosine, adenine and guanine as well as the sugar-phosphate subunits. Guanine, once hydrolyzed from the nucleic acid, will no longer remain in solution (Online Lab Manual, 2023).

(d) **The tube labelled "unhydrolyzed nucleic acids"**

As no hydrolysis of nucleic acids took place, there would be no base subunits in the tube, only whole nucleic acid polymers.

If the hydrolysis steps carried out last week were only partially successful, what substances or molecules would you expect to be present in:

(a) **The tube labelled "hydrolyzed protein"**

A mixture of proteins and amino acids found in yeast cells would be present.

(b) **The tube labelled "hydrolyzed nucleic acids"**

A mixture of partially broken down nucleic acid polymers, nucleotide molecules, and individual base and sugar-phosphate subunits would be present.

Into what category of substances do alanine, histidine, aspartic acid, lysine, and methionine fall? What about adenine, cytosine, and uracil?

Alanine, histidine, aspartic acid, lysine and methoinine are amino acids. Adenine, cytosine and uracil are nitrogenous bases of nucleic acids.

References

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Perrett, D. and Nayuni, N. (2014). 23 - efficacy of current and novel cleaning technologies (proreveal) for assessing protein contamination on surgical instruments. In Walker, J., editor, *Decontamination in Hospitals and Healthcare*, pages 598–619. Woodhead Publishing.

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