

A ¹H-NMR Study of Water Soluble Metabolites from Bread Yeast as a Model for Learning Bioinformatics and Metabolomics

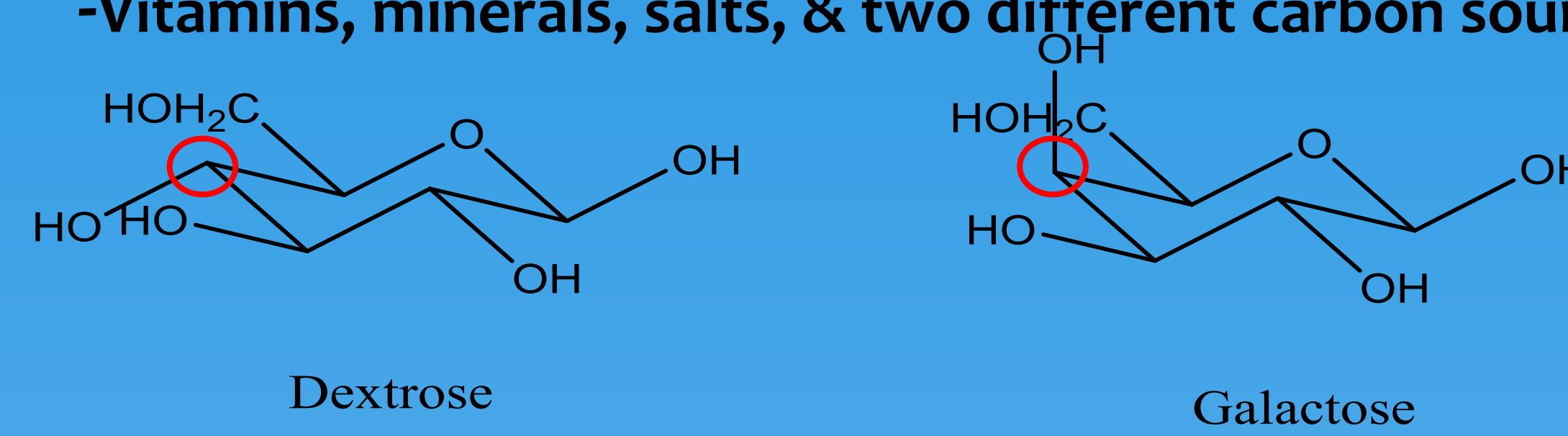
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ABSTRACT

Yeast cells, *Saccharomyces cerevisiae*, are microorganism essential for bread and beer production. Yeast cells have the capacity to utilize various pathways for the conversion of simple sugar molecules into CO₂ and alcohol. Using metabolome-base research we characterized water soluble metabolites from *S. cerevisiae* using two different sugars dextrose and galactose. These metabolites were characterized by one-and two-dimensional NMR. We expect to see a difference in metabolome between yeast grown in galactose and those grown in dextrose. NMR will identify the small molecule metabolites and the YMDB database will assist us in identifying the frequency of their presence in the analyte. COSY-NMR is used to interpret correlation differences between functional groups in metabolite structures. ¹H-NMR –based profiling identified the metabolites as products produced from *S. cerevisiae* organisms. The chemical-peak-shift values were integrated into a bioinformatics data base for metabolite-profiling analysis.

METHODS

- Yeast Cell Inoculation**
-Vitamins, minerals, salts, & two different carbon sources
- Lyse Yeast Cells**
-Centrifuge, alcohol extraction, vortex, & water sublimation
- COSY-NMR Analysis**
- ¹H-NMR Analysis**
-Experiment taken at 100 scans
- Yeast Metabolome Database**



RESULTS

Yeast Metabolome Database

Norleucine	131.1729	<chem>CCCCC(N)C(=O)O</chem>	41/42
327-57-1	C ₆ H ₁₃ NO ₂		
Glucosamine 6-phosphate	259.151	<chem>OC[C@H]1O[C@@H](COP(=O)(O)O)[C@H](O)[C@@H](O)[C@H]1O</chem>	39/40
3616-42-0	C ₆ H ₁₄ NO ₈ P		
Glucose 6-phosphate	260.1358	<chem>OC[C@H]1O[C@@H](COP(=O)(O)O)[C@H](O)[C@@H](O)[C@H]1O</chem>	32/33
56-73-5	C ₆ H ₁₃ O ₉ P		

ymdb.ca

A

B

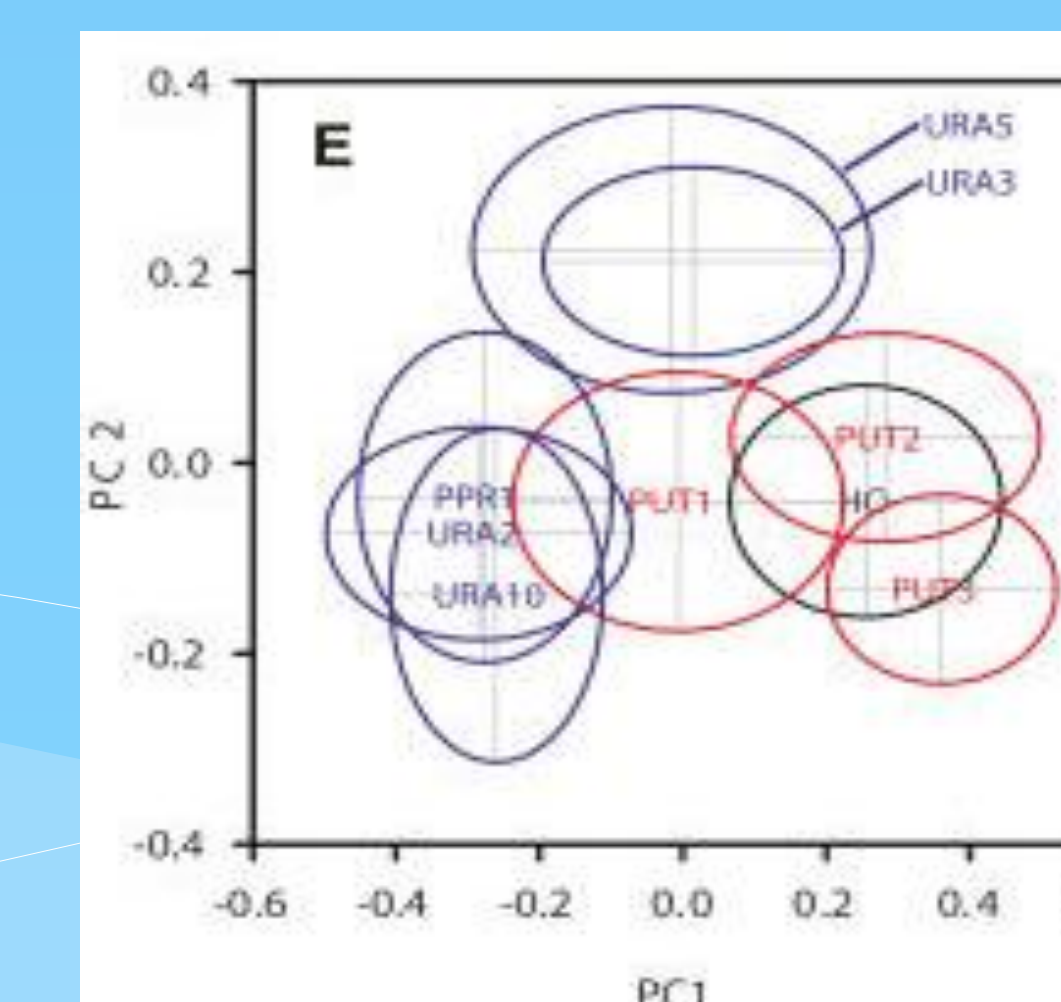
Figure 3. Chemical shift values from ¹H-NMR entered into yeast metabolome database for metabolomic profiling. (A) The metabolites—norleucine, glucosamine-6-phosphate, glucose-6-phosphate, & many more metabolites (data not shown)—analyzed as intermediates or products from the glycolytic pathway. Ratio of chemical shift values (green & red circles) for norleucine. (B) Norleucine has 42 unique chemical shift values, which is a value indicated in the red circle (A). The green circle (A) represents the number of peak matches in our scan to the peaks in YMDB.

CONCLUSIONS

- These results may provide possible metabolite dissimilarities produced from cells cultured in dextrose and galactose media.
- The 1-dimensional spectrum show metabolites differ in the aromatic region.
- NMR and YMDB are not enough to determine the difference between the metabolomes.

Future Work

- Use PCA to determine relative relationship and significance between metabolomics.
- Identify outliers of multivariable data sets.

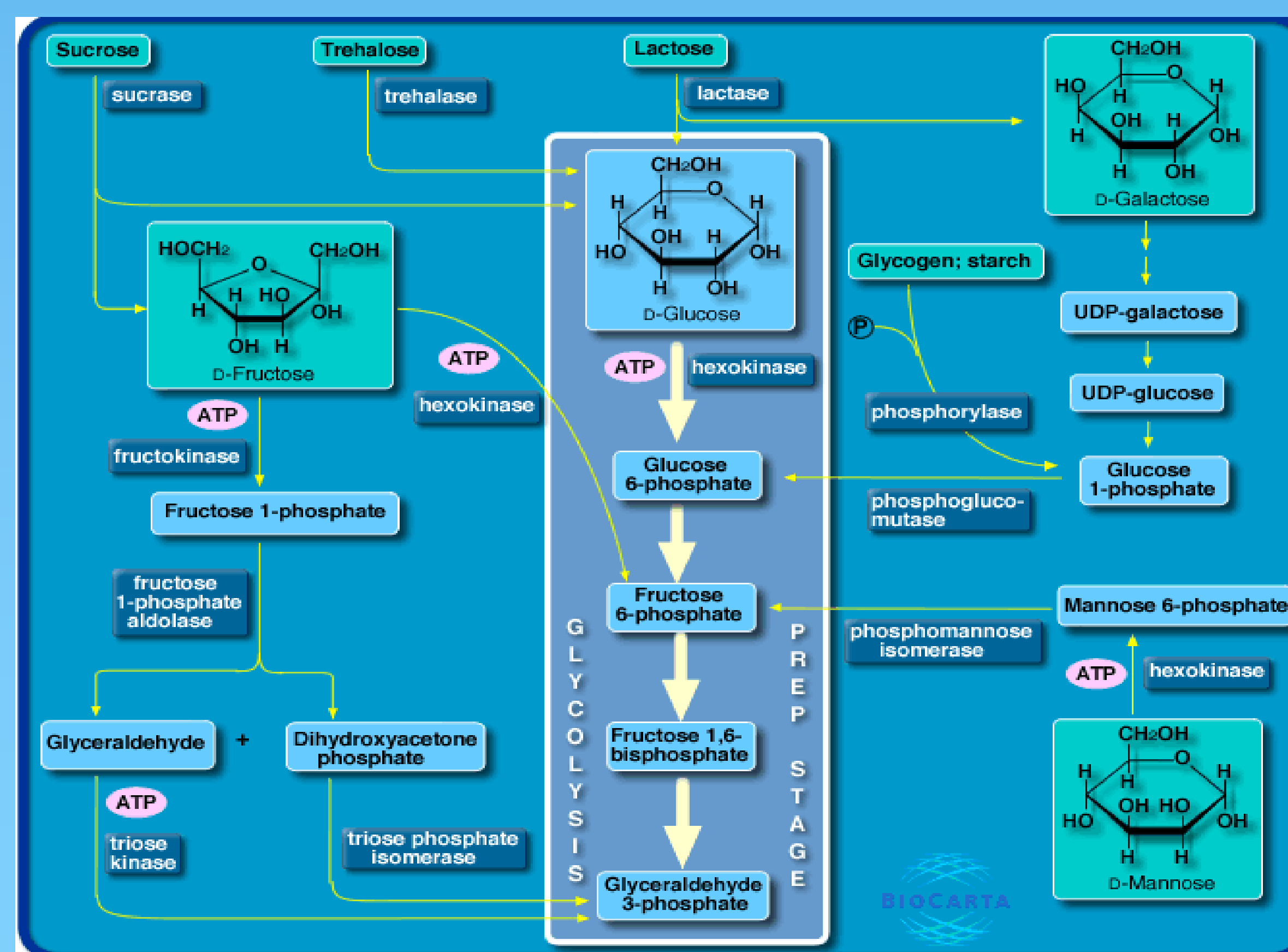


Bundy JP, et al. 2007. Evaluation of predicted

References

Bundy JG, Papp B, Harmston R, Clayton EM, Burton N, Reesce RJ, Oliver SG, and Brindle KM. Evaluation of predicted network modules in yeast metabolism using NMR-based metabolite profiling. *Genome Res.* 17(4): 510-19, 2007.

The Glycolytic Pathway



<http://www.biocarta.com/pathfiles/feederpathway.asp>

Figure 1. A modeled pathway yeast cells use to metabolize carbon sources for energy purposes. Metabolites may be produced as intermediates or products to drive other thermodynamically-favored reactions.

***Aim of the study: To identify and quantify metabolites present in *S. Cerevisiae* from two different carbon sources dextrose and galactose.**

RESULTS

NMR Spectra

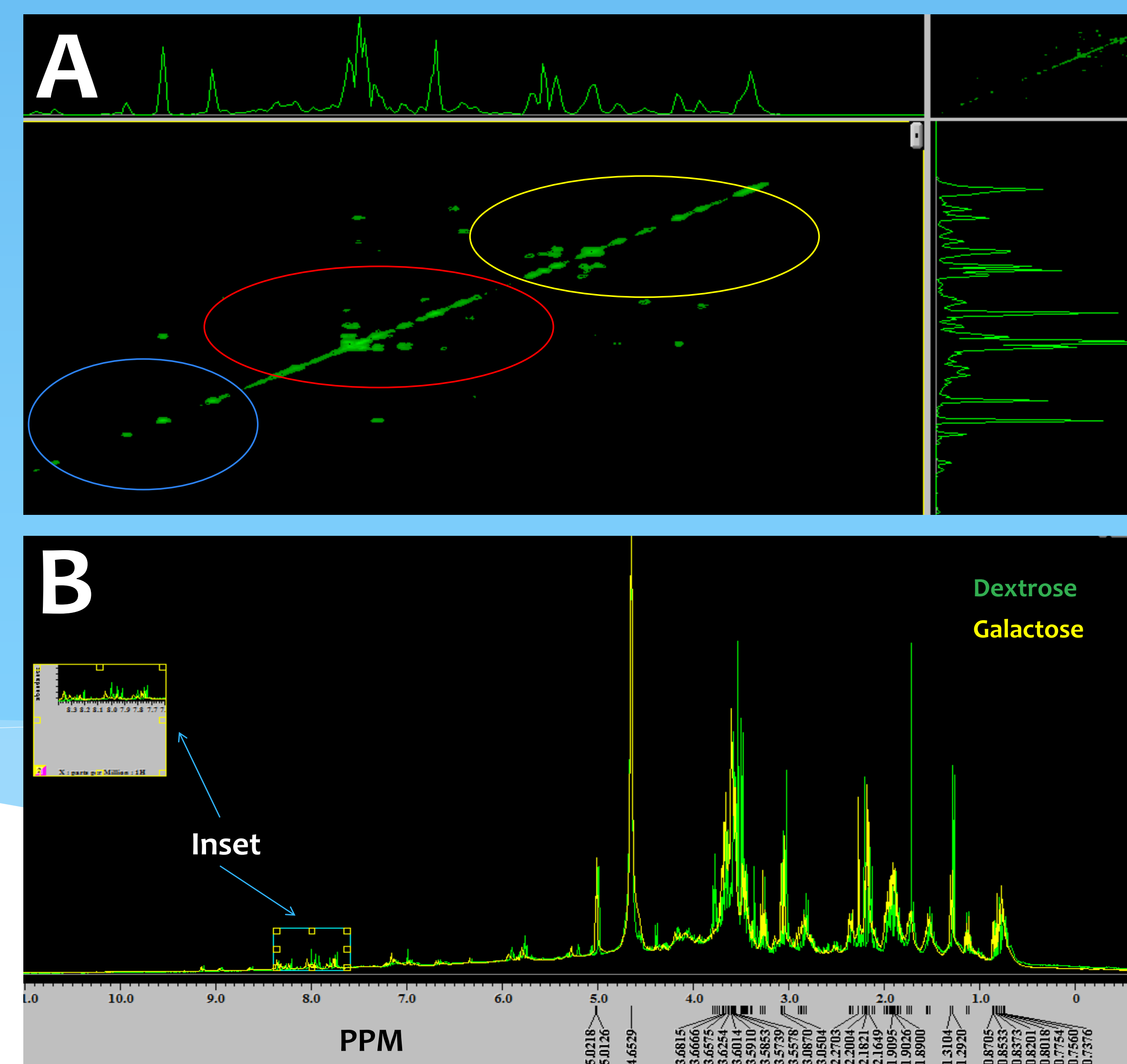


Figure 2. (A) COSY spectra of Dextrose fixed at a lower bias. The two-dimensional correlation patterns characterize the presence of unknown metabolites in these different regions: (blue) α-carbon region, (red) alcohol region, & (yellow) aliphatic region. (B) ¹H NMR spectra overlapped to demonstrate the differences between dextrose (green) and galactose (yellow). Inset shows differences in the aromatic region.