LC-MS/MS Detection of Monosaccharides from Yeast Selected for Xylose Consumption

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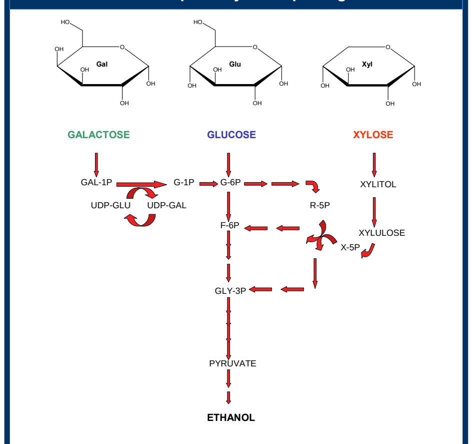
Overview

An LC-MS/MS method was developed to demonstrate the separation and identification of monosaccharides in complex sample matrices. Concentrations of simple sugars were quantified by LC-MS/MS using multiple reaction monitoring (MRM) mode. This method was applied to a biological system, measuring xylose consumption by yeast strains which were selected for efficient ethanol production.

Introduction

- Increasing dependence on petroleum-based fuel heightens the need to develop alternative, cost-effective energy sources. Yeast strains capable of producing ethanol from simple sugars provide a promising avenue toward this end.
- Efficient ethanol production requires the ability to ferment sugars from cellulose- and hemicellulose-containing plant materials. In contrast to fermentation of glucose, the building-block of cellulose, the fermentation of hemicellulose constituents (e.g. xylose and other pentose sugars) remains elusive.
- *S. cerevisiae*, a strain commonly employed for the production of ethanol from hexose sugars such as glucose and galactose, is currently incapable of incorporating pentose sugars into its metabolome.[1]

SCHEME 1. Metabolic pathway of simple sugars to ethanol.

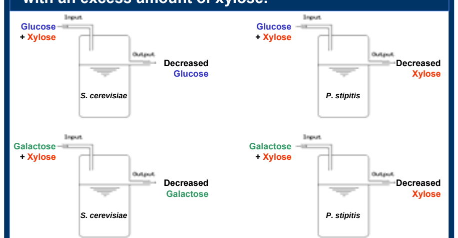


- An alternate strain, *P. stipitis*, which is known to selectively utilize xylose under aerobic conditions is present as a control to monitor xylose concentration over time. The challenge remains to reproduce this activity anaerobically, a necessary condition, for efficient ethanol production by *S. cerevisiae*.

Methods

- Yeast strains, including *S. cerevisiae* and *P. stipitis* (control), were grown in a chemostat, an apparatus used to provide cell cultures with a continuous supply of nutrients (as shown in Scheme 2), under limited glucose or galactose and excess xylose concentrations, 0.08% and 1% w/v respectively.
- Effluent from the chemostats was sampled periodically and analyzed for sugar consumption. The effluent consists mainly of growth media containing water, salts, vitamins and minerals, in addition to the glucose or galactose and xylose.

SCHEME 2. Chemostats containing yeast strains and low levels of glucose or galactose are supplemented with an excess amount of xylose.



Sample Preparation: Protein precipitation was performed by adding 300μL of methanol to 100μL of 600x diluted effluent collected from the chemostats. Samples were then centrifuged and the supernatant was dried and resuspended in 100μL of 60:40, ACN:H_2O buffer solution.

Quantitation Assays: Commercially available glucose, xylose and galactose were used for method development and as external standards for construction of calibration curves. Detection of analytes was performed in MRM mode, using the transitions 179 to 89, 149 to 89, and 179 to 89 m/z for glucose, xylose and galactose quantitation, respectively (see Figure 1). All samples analyzed contained either xylose and glucose or xylose and galactose mixtures, as the diastereomers glucose and galactose are indistinguishable by LC-MS/MS.[2]

Instrumentation: All analyses were carried out by electrospray LC-MS/MS using an Agilent 1100 HPLC and Micromass Quattro Premier triple quadrupole mass spectrometer. QuanLynx software was used for data analysis. HPLC conditions: C18 Luna Amino 150 x 2.1mm, 5μm particle size; 5 minute isocratic analysis, 60% B; A: water, B: acetonitrile; 200ml/min flow rate.

Results

FIGURE 1. MS/MS spectra of monosaccharide analytes. Transitions selected for MRM monitoring include 179.0 to 88.8, 148.9 to 88.7 and 179.1 to 88.9 m/z for glucose, xylose and galactose, respectively.

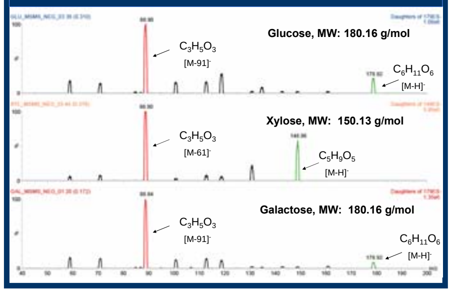


FIGURE 2. Monosaccharide quantitation assay. The response was linear over a range of 0.5-1,000 pmol; LOQ ≈ 0.3, 6.5 and 0.3 pmol; and LOD ≈ 0.1, 2.5 and 0.1 pmol; for glucose, xylose and galactose, respectively.

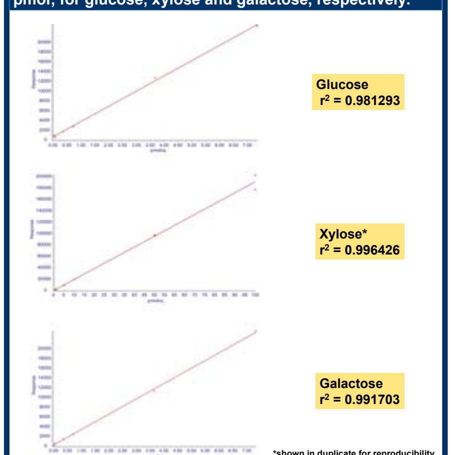
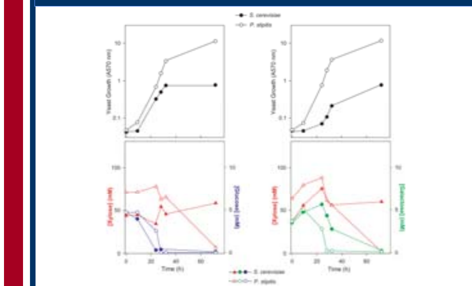


FIGURE 3. Quantitation of sugar consumption. The concentrations of glucose, xylose and galactose in the growth media over time are indicative of the yeasts' ability to metabolize these various monosaccharides.



Conclusions

- Amine-bonded reverse phase column provides enhanced sugar separation and ideal retention time of ~2.5 minutes under isocratic mobile phase conditions.
- MRM detection of unique fragmentation transitions permits accurate quantitation for structurally related compounds.
- Broad linear response range of monosaccharides enables dilution of samples into buffer solution, eliminating matrix effects of the biological medium.

Future work

- Perform extended protein precipitation assays to determine an optimal sample recovery procedure.
- Monitor intermediate compounds in the metabolic pathway of ethanol production from simple sugars by LC-MS/MS to identify fermentation stages.
- Explore *S. cerevisiae* dependence on glucose via introduction of alternate sugar-based carbon sources to determine ideal growth conditions for xylose consumption.

References

1. Jeffries, T., *Biotechnology*, 17, 1-7, 2006.
2. Clarke, M. et al., *J. Agric. Food Chem.*, 54, 1975-1981, 2006.

Acknowledgments

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