Development of computational and experimental methods for measuring biomass composition and evaluating its impact in genome-scale models predictions

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INTRODUCTION

Nowadays, an indispensable tool for the study of metabolic systems biology is the genome-scale metabolic network reconstruction. An important part of a metabolic model is the biomass equation since this reaction will ultimately determine the predictive capacity of the model in terms of essentiality and flux distributions. Thus, in order to obtain a reliable metabolic model, the biomass precursors and their coefficients must be as precise as possible. Ideally, determination of the biomass composition would be performed experimentally, but when no experimental data are available this is established by approximation to closely related organisms. Computational methods however, can extract some information from the genome such as amino acid and nucleotide compositions .

Objective: Determine and evaluate how biomass precursor coefficients affect the predictability of genome-scale metabolic models.

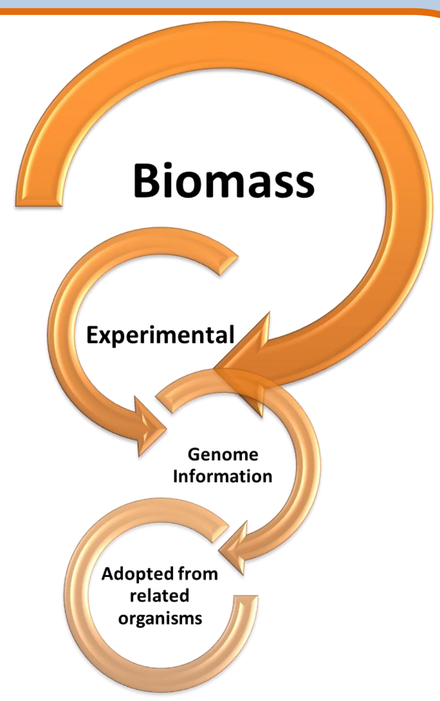
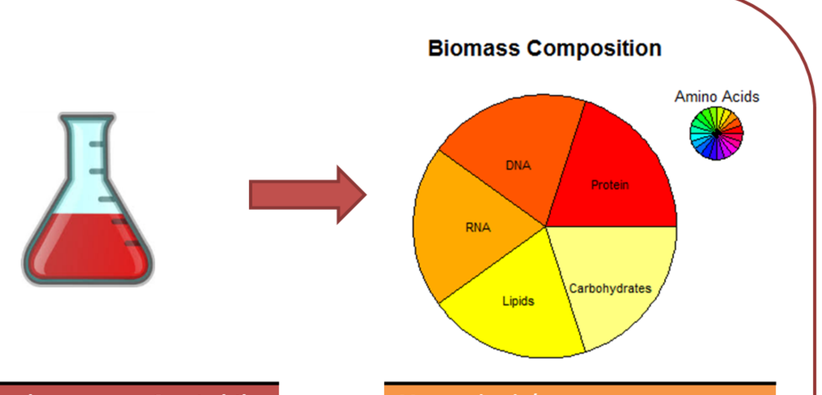


Figure 1. Diagram representing the methods used for formulating the biomass composition

METHODOLOGY

Experimental



Organisms	Caracteristics
Bacillus subtilis	Gram + bacteria
Corynebacterium glutamicum	Gram + bacteria
Enterococcus faecalis	Gram + bacteria
Escherichia coli	Gram - bacteria
Kluyveromyces lactis	Yeast
Pseudomonas putida	Gram - bacteria
Saccharomyces cerevisiae	Yeast
Salmonella typhimurium	Gram - bacteria

Macromolecule/ Building block	Method
Protein	Biuret
DNA	Fluorescence with DAPI
RNA	Cold percloric acid
Lipids	Phosphovanillin
Carbohydrates	Phenol sulphuric
Amino Acids	HPLC with OPA and FMOC derivatization

Computational

Development of a Java tool for estimation of biomass coefficients from genome information (*in silico*)

Incorporate experimental and *in silico* biomass coefficients in genome-scale metabolic models

Evaluation of the impact of different biomass compostions in metabolic model simulations, using Optflux 3.2.8

RESULTS

Macromolecules

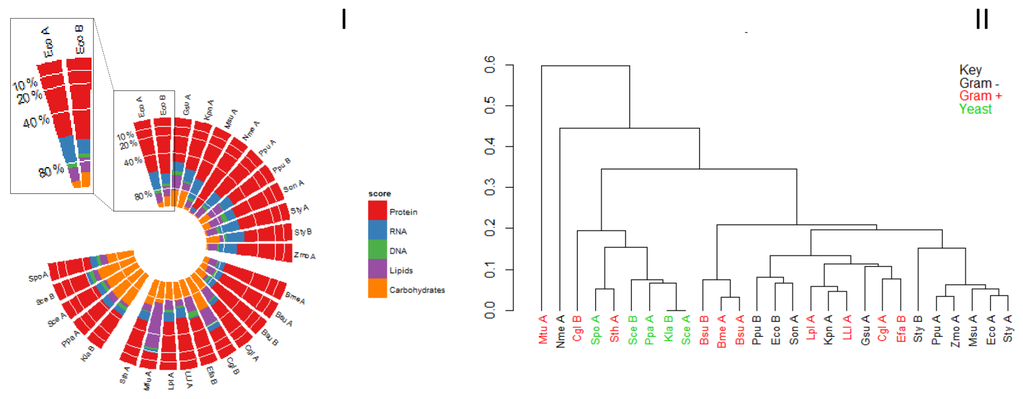


Figure 2. Comparison of macromolecular biomass compositions determined experimentally. Literature experimental data (A) and our experimental data.
(I) Polar histogram plot representing biomass macromolecular compositions for several organisms by species (gram negative bacteria, gram positive bacteria and yeast). **(II)** Hierarchical Clustering using the parameters euclidean distance and average method.

Amino Acids

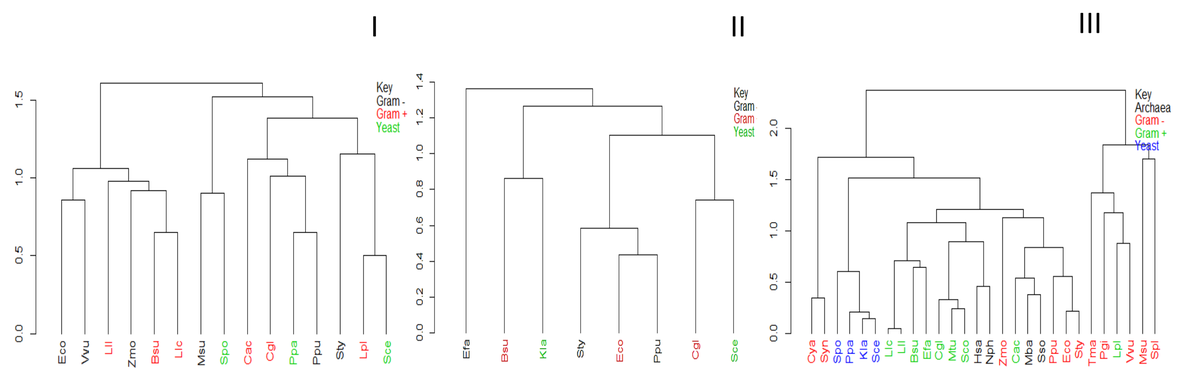


Figure 3. Statistical analysis of amino acid composition. Hierarchical Clustering using the parameters euclidean distance, average method. Pearson correlation was applied to all data. **(I)** Hierarchical Clustering for literature experimental data. **(II)** Hierarchical Clustering for our experimental data. **(III)** Hierarchical Clustering for *in silico* data.

In silico Simulations

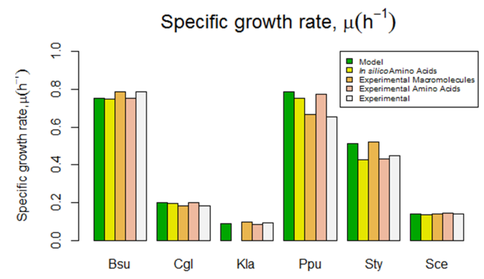


Figure 4. Specific growth rate predictions when changing biomass coefficients (amino acids and/or macromolecules) in genome-scale metabolic models. Abbreviations: Bsu – *B. subtilis*; Cgl – *C. glutamicum*; Kla – *K. lactis*; Ppu – *P. putida*; Sty – *S. thyphimurium*; Sce – *S. cerevisiae*.

CONCLUSIONS

- The results obtained suggest that the macromolecular composition is conserved among related organisms;
- Experimental data for amino acid composition seem to have no similarities for related organisms;
- The impact of macromolecular composition on specific growth rates and flux distributions is larger than the impact of amino acid composition, even when data from closely related organisms are used.

ACKNOWLEDGMENTS

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ERA IB

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