Exploring methane mitigation strategies in photosynthetic microorganisms through genome-scale metabolic models

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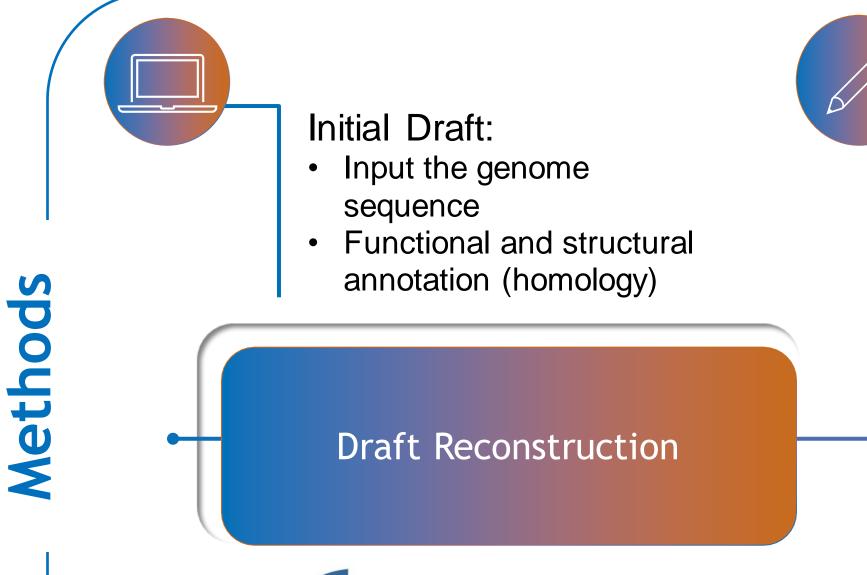
The problematic of greenhouse gas (GHG) emissions is a global environmental challenge that has raised concerns in the past few decades. Particularly, the increase in atmospheric concentrations of carbon dioxide, methane, and other damaging gases can lead to catastrophic repercussions to life as we know it. Therefore, reducing GHG emissions and fomenting strategies for their mitigation are crucial steps that need to be taken in order to meet the Paris Agreement and ultimately ensure a sustainable future for our planet and those that inhabit it [1]. Taking this into account, our work aims at identifying the metabolic capabilities of photosynthetic microorganisms to reduce methane emissions.

Genome-scale metabolic (GSM) models allow the in silico simulation and prediction of metabolic fluxes on a large scale, providing a powerful tool for optimizing and designing metabolic engineering methods. By integrating high-throughput data with GSM models, a comprehensive understanding of cellular metabolism and identification of strategies to improve a certain objective function can be obtained. The importance of this emerging technology in industry stems from its ability to offer a faster and more cost-effective approach, surpassing the efficiency of traditional methods [2].

Herein, we describe the reconstruction of GSM models for the microalga Chlorella vulgaris sp. - iGA1305 -, and for the cyanobacterium Synechocystis sp. - iJG708. Both GSM models provide a powerful tool for metabolic improvement, allowing predictions and simulations of CH₄ metabolism in response to different culture conditions and genetic modifications.

Both C. vulgaris and Synechocystis already have GSM models. So, why should we reconstruct a model for an organism that already has one?

- Genomic information is continuously updated on databases.
- Previous models don't incorporate methane in their reconstruction.



the C. reinhardtii model (iRC1080) shown for comparison

Reversibility and Directionality

- Transport Reactions
- Mass Balance
- Pathway-by-pathway analysis
- Compartmentalization

Growth medium and exchange reactions

- Definition of constraints
- Biomass and Energy Requirements

Conversion into a

- In silico Simulations Topology Analysis
- Comparison with experimental data/other models

Model Validation

Gene essentiality

Manual Curation

BRENDA **METACYC** Merlin

stoichiometric model







Chlorella vulgaris – iGA1305

Model Overview

Table 1: Experimental and predicted growth rates reported for *C. vulgaris* (iGA1305/iCZ843) and *C. reinhardtii* (iRC1080) shown for comparison

| Conditions | <i>i</i> GA1305 (This work) | <i>i</i> CZ843 | | <i>i</i> RC1080 | |
|-----------------|--------------------------------|--------------------|--------------------------|--------------------|--------------------------|
| | Growth rate | Growth rate | Experimental | Growth rate | Experimental |
| Photoautotrophy | 0.103 | 0.025 | 0.014–0.025 ^a | 0.154 | 0.035-0.09b |
| Heterotrophy | 0.023 ^c | 0.017 ^c | 0.018–0.025 ^a | 0.029 ^d | 0.059–0.084 ^b |
| Mixotrophy | 0.127 ^c | 0.041 ^c | 0.02–0.03 ^a | 0.182 ^d | 0.066 ^b |

^a Data from [3]. ^b Data from [4]. ^c glucose used as organic carbon source. ^d Acetate used as organic carbon source.

Table 2: Properties of the GSM model for C. vulgaris (iGA1305), for the previous Chlorella model (iCZ843) and for

| Organism | Model | Genes | Reactions | Metabolites |
|-------------------------|-----------------|-------|-----------|-------------|
| C. vulgaris (This work) | <i>i</i> GA1305 | 1305 | 2659 | 2027 |
| C. vulgaris (UTEX-395) | <i>i</i> CZ843 | 843 | 2286 | 1770 |
| C. reinhardtii | <i>i</i> RC1080 | 1080 | 2191 | 1706 |

Synechocystis – iJG706

Model Overview

Table 3: Experimental and predicted growth rates reported for *Synechocystis* shown for comparison

| Conditions | iJG706 (This work) | iJN678 [7] | |
|-----------------|-----------------------|--------------------|--------------|
| | Growth Rate | Growth Rate | Experimental |
| Photoautotrophy | 0.085 | 0.088 | 0.085 [5] |
| Heterotrophy | 0.063 | 0.064 | 0.076 [6] |
| Mixotrophy | 0.043 | 0.056 | 0.059 [6] |

Table 4: Properties of the GSM model for Synechocystis (iJG706), for the previous Synechocystis models (iJN678, iSyn811 and iSyn669) shown for comparison

| Organism | Model | Genes | Reactions | Metabolites |
|---------------------------|-------------|-------|-----------|-------------|
| Synechocystis (This work) | iJG706 | 706 | 2172 | 1619 |
| Synechocystis | iJN678 | 678 | 863 | 795 |
| Synechocystis | iSyn811 [8] | 811 | 956 | 911 |
| Synechocystis | iSyn669 [9] | 669 | 882 | 790 |

- The reconstruction process did not reveal any genomic evidence of enzymes associated with methane metabolism
- Both models were validated regarding spontaneous growth, growth rates in different conditions, auxotrophies and uptake/secretion values
- The models are subject to change as new approaches are employed towards fine-tuning
- Next steps will involve the incorporation of possible methane-utilizing pathways in the models
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