

# A chemically-defined growth medium for a model human gut microbiome

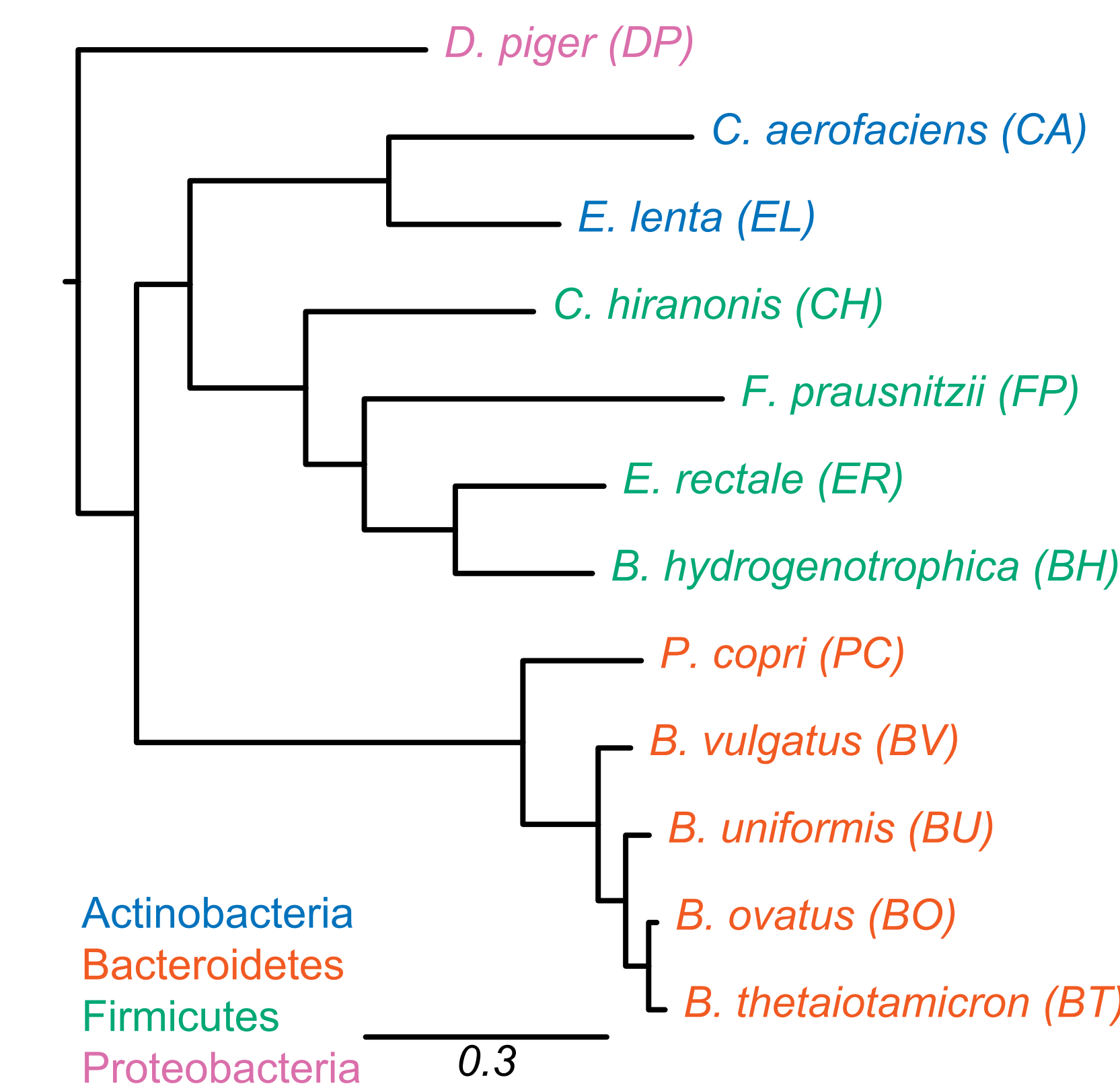
Joshua J. Hamilton<sup>1</sup>, Ryan L. Clark<sup>1</sup>, Emma S. Groblewski<sup>1</sup>, and Ophelia S. Venturelli<sup>1,2,3</sup>

<sup>1</sup>Department of Biochemistry, <sup>2</sup>Department of Bacteriology, <sup>3</sup>Department of Chemical and Biological Engineering, University of Wisconsin-Madison

## A MODEL HUMAN GUT MICROBIOME

Microorganisms rarely exist in isolation, but instead form complex communities, or microbiomes. Interactions among microbes are ubiquitous in these communities, and microbial interactions are major determinants of microbiome function. These interactions are especially important in the human gut, where the microbiome plays diverse roles, including preventing disease and enhancing digestion. In-vitro, culture-based model microbiomes are necessary to interrogate the mechanisms of interspecies interaction.

We previously developed a model microbiome containing 12 species that together encompass the functional and phylogenetic diversity of the natural human gut microbiome<sup>1</sup>. Here, we present a chemically defined culture medium that supports the growth of 11 of 12 members of this community. Growth experiments on a series of defined media enabled us to characterize the nutritional preferences and biosynthetic capabilities of these microbes, as well as identify specific metabolites that enhance or inhibit growth.



## PROSPECTUS

Development of a chemically-defined medium enables mechanistic investigations of interspecies interactions, such as identifying cross-fed metabolites.

Media composition can be used to alter monospecies abundance, and possibly control community composition.

Our defined medium serves as a resource for refinement of genome-scale, computational metabolic models.

## ACKNOWLEDGEMENTS

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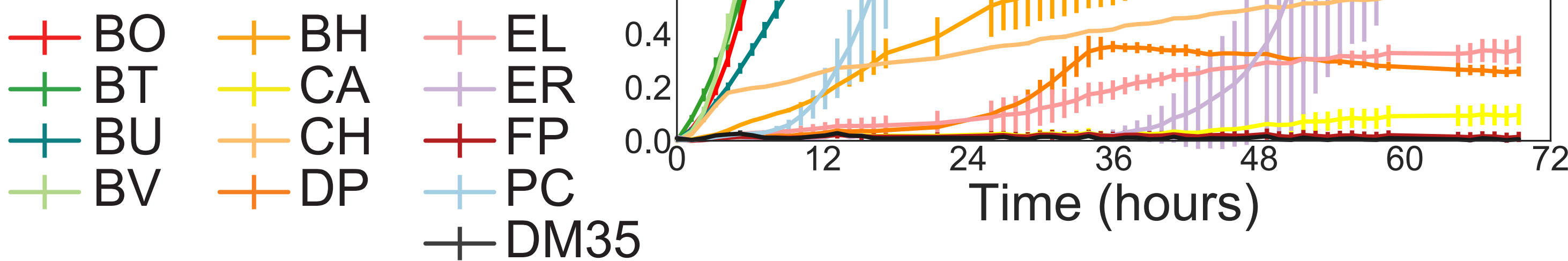
## REFERENCES

1. Venturelli OS, Carr AC, Fisher G, Hsu RH, Lau R, et al. (2017). bioRxiv.  
2. Heinken A, Khan MT, Paglia G, Rodionov DA, Harmsen HJM, et al. (2014). J Bacteriol 196: 3289–3302.

## DEVELOPING A CHEMICALLY-DEFINED GROWTH MEDIUM (DM35)

Media	Component Added	BO	BT	BU	BV	BH	CA	CH	DP	EL	ER	FP	PC
DM0													
DM3	Amino acid mixture												
DM4	Xanthine, Pyridoxal												
DM6	Ammonium chloride												
DM10	Increased amino acids												
DM14	MOPS												
DM16	Vitamin mixture												
DM20	Biotin												
DM21	Increased amino acids												
DM22	Increased vitamins												
DM23	Calcium chloride												
DM24	Copper sulfate												
DM33	Inositol, Tween, Lactate, Magnesium sulfate												
DM34	Arabinose, Maltose, Glucose												
DM35	Remove Tween												

We began with a media previously shown to support the growth of the beneficial human gut microbe *Faecalibacterium prausnitzii*<sup>2</sup>. Additional components were added based on literature review and *in-vitro* growth screens. The final medium supported growth of 11 of 12 monospecies in our model microbiome, as measured by the maximum OD<sub>600</sub> of three biological replicates after 72 hours.



## MODELING THE EFFECT OF MEDIA COMPOSITION ON GROWTH

DM35 contains all 20 amino acids and four sugars. All monospecies were grown in titrations of amino acid and sugar mixtures for 48 hours. A growth model was fit to the data, and linear regression was used to explore how the growth rate and carrying capacity of each monospecies change with media composition.

Growth model:  $\frac{dx}{dt} = x(\mu - \alpha x)$

$x$  : abundance

$\mu$  : growth rate

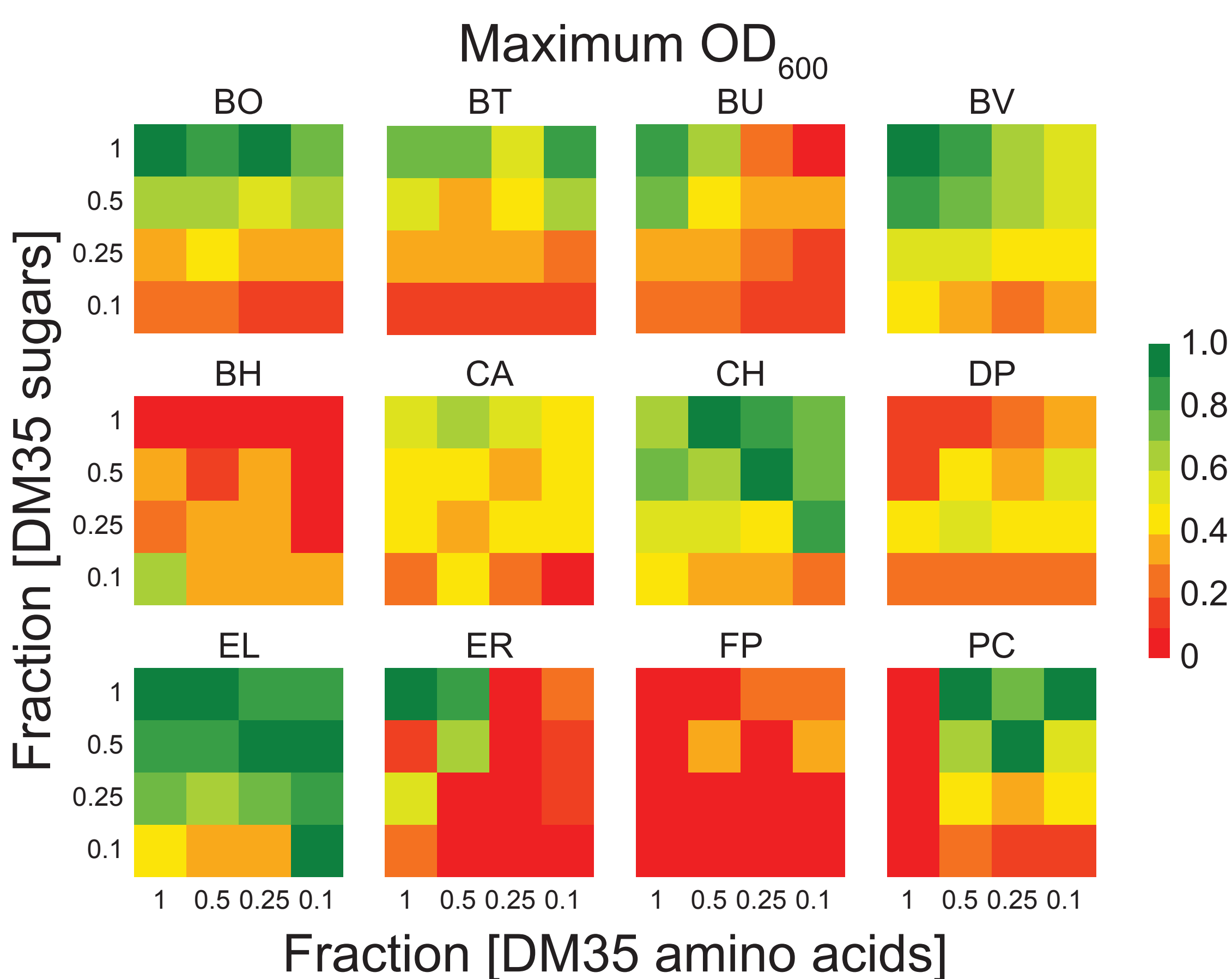
$\alpha$  : self-interaction term

At steady-state:  $x_{\text{steady state}} = \frac{\mu}{\alpha}$  = carrying capacity

Regression model:  $x_{\text{steady state}} = \beta f_s + \gamma f_{aa} + x_0$   $f_s$  : fraction of [DM35 sugars] (0 to 1)

$\mu = \epsilon f_s + \zeta f_{aa}$   $f_{aa}$  : fraction of [DM35 amino acids] (0 to 1)

$x_0$  : initial abundance



### Model results for carrying capacity

	$\beta$	$\gamma$	$x_0$	$R^2$
BO	0.64	0.04	0.11	0.91
BT	0.49	0.05	0.08	0.88
BU	0.37	0.29	-0.02	0.60
BV	0.35	0.19	0.25	0.75
BH	0.35	-0.37	0.13	0.65
CA	0.28	-0.12	0.28	0.64
CH	0.50	0.02	0.33	0.78
DP	0.43	-0.10	-0.11	0.18
EL	0.44	0.05	0.39	0.68
PC	0.44	0.64	-0.64	0.69

### Model results for growth rate

	$\epsilon$	$\zeta$	$R^2$
BO	0.90	2.71	0.62
BT	-0.25	1.35	0.46
BU	-0.90	2.42	0.61
BV	0.40	4.65	0.56
BH	1.12	0.21	0.68
CA	0.02	0.27	0.71
CH	0.47	0.25	0.73
DP	0.43	0.03	0.74
EL	0.36	0.46	0.55
PC	0.44	-0.02	0.51