A Multi-level "-Omics" Approach to the Study of the Growth Regulation of Methanotrophs

Phillip Sun[1], Catherine Tays[1,2], Fabini D. Orata[1,2], Melissa Harrison[2], Lisa Y. Stein[1], and Dominic Sauvageau[2]

BACKGROUND

- Methane is a common industrial waste and potent greenhouse gas that can serve as a feedstock for methanotrophic bacteria. These bacteria can use methane to produce value-added products such as biofuels.
- A thorough understanding of the physiology and regulation of methanotrophic bacteria is imperative, including a more narrow focus on industrially relevant strains to analyze regulatory effects of further media refinement (i.e., nutrients available, copper concentration, acidic conditions, etc.).
- The multi-level "-omics" approach will demonstrate how different growth conditions affect the structure, function, and metabolism of the different species, leading to a more efficient optimization process for the production of the biofuels (alcohols, isoprenoids), biofuel precursors (isoprene), and other value-added products.

AIMS AND OBJECTIVES

Growth optimization
- AMS + CH_4
- AMS + MeOH
- NMS + CH_4
- NMS + MeOH
- varying [copper]

Genomic inventory of relevant genes
- Whole-genome sequencing
- presence/absence of relevant genes

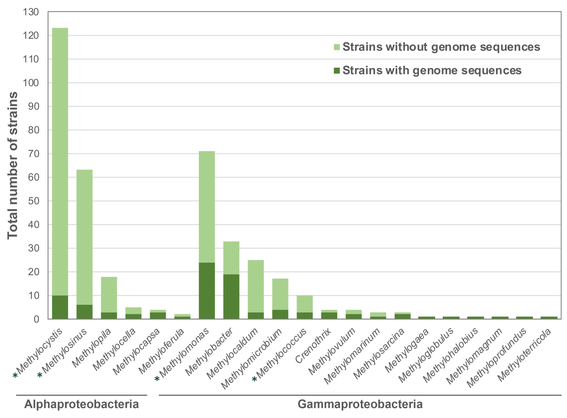
Methanotroph strains

Global RNA regulation
- RNA sequencing
- carbon flux
- bioproduction pathways

Metabolomic analysis
- nutrient effects
- value-added products (secondary metabolites)

RESULTS

Genome sequence availability for methanotrophs



Number of methanotrophic strains in different genera of alpha- and gamma-proteobacteria. Although hundreds of strains have been identified, only a few have sequenced genomes. Other industrially significant strains may have been overlooked.

Upregulated and downregulated genes for methane oxidation in varying conditions

Methane → Methanol

	A	B	C	D	E
methane, monooxygenase	1.08	-1.97	-1.69	-1.45	2.07
methane, monooxygenase 2	-1.27	-1.41	-2.02	1.59	1.83
methane, monooxygenase 3	1.03	-2.80	-6.32	2.55	7.33
methane, monooxygenase 4	1.14	-2.77	-6.25	2.56	8.14
ammonia, monooxygenase	-1.22	-2.12	-7.02	3.17	6.43
ammonia, monooxygenase 2	-1.37	-2.24	-6.94	3.00	6.14
methane, monooxygenase 5	-1.19	-1.70	-5.63	2.74	5.50
methane, monooxygenase 6	-1.33	2.78	1.04	3.31	-1.51
methane, monooxygenase 7	-2.50	-1.47	-1.97	1.17	-1.70

Methanol → Formaldehyde

	A	B	C	D	E
methanol, dehydrogenase	-1.12	1.05	1.07	-1.01	-1.18
methanol, dehydrogenase 2	-1.28	-1.03	-3.40	3.18	2.93
methanol, dehydrogenase 3	-1.40	1.27	-4.54	4.48	3.61
methanol, dehydrogenase 4	-1.04	1.63	-1.27	2.82	1.29
methanol, oxidase	-1.05	1.12	1.25	-1.10	-1.41
methanol, dehydrogenase 5	-3.42	-1.76	-3.14	1.54	-1.46

Formate → Carbon Dioxide

	A	B	C	D	E
formate, hydrogenlyase	-1.23	1.23	1.63	-1.25	-2.62
formate, dehydrogenase	1.42	1.36	-1.12	1.64	1.76
formate, dehydrogenase 2	1.48	1.17	1.06	1.11	1.50
formate, dehydrogenase 3	1.35	1.49	1.20	1.29	1.14
formate, dehydrogenase 4	1.41	1.51	1.72	-1.09	-1.22
formate, tetrahydrofolate ligase	1.16	1.86	1.33	1.54	-1.15
formate, dehydrogenase 5	1.49	1.33	-1.07	1.53	1.78
formate, dehydrogenase 6	1.47	1.70	2.20	-1.21	-1.46
formate, dehydrogenase 7	-2.91	-1.44	-3.26	2.17	-1.05

Formaldehyde → Formate

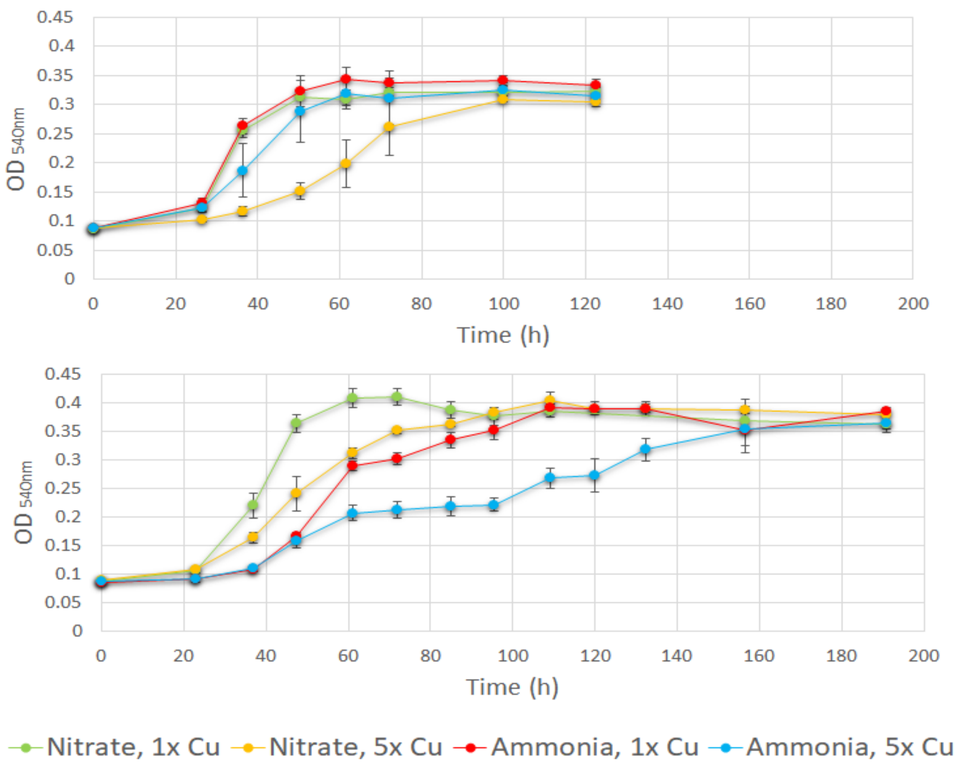
	A	B	C	D	E
H4MPT-linked, C1, transfer, pathway, protein	1.38	1.17	1.99	-1.71	-1.70
formylmethanofuran--tetrahydromethanopterin, formyltransferase	1.46	-1.00	-1.22	1.22	2.04
tetrahydromethanopterin, synthesis, protein	1.03	1.01	1.29	-1.29	-1.39
methylenetetrahydromethanopterin, dehydrogenase	1.24	1.24	1.36	-1.08	-1.10
N(5)%2CN(10)-methenyltetrahydromethanopterin, cyclohydrolase	1.10	1.09	-1.01	1.14	1.16
formylmethanofuran, dehydrogenase	1.37	-1.03	1.28	-1.39	1.12
formylmethanofuran, dehydrogenase 2	1.21	-1.04	-1.36	1.31	1.78
formylmethanofuran, dehydrogenase 3	1.41	-1.01	-1.13	1.10	1.81
5-formyltetrahydrofolate, cyclo-ligase	1.26	1.08	2.30	-2.17	-2.18
folate-binding, protein	-1.08	1.15	1.20	-1.01	-1.49
5%2C10-methylenetetrahydrofolate, reductase	1.34	-1.24	-3.07	2.34	6.52
formate--tetrahydrofolate, ligase	1.16	1.86	1.33	1.54	-1.15
methylenetetrahydrofolate, dehydrogenase	1.47	1.45	1.44	1.06	1.04
methenyltetrahydrofolate, cyclohydrolase	1.47	1.16	1.23	-1.04	1.28
aldehyde-activating, protein	-1.14	1.02	-1.48	1.56	1.40
aldehyde-activating, protein 2	1.04	1.02	-1.09	1.13	1.18
aldehyde-activating, protein 3	-1.11	1.06	1.07	1.01	-1.27
aldehyde-activating, protein 4	-1.05	-1.06	-2.17	2.03	2.05
aldehyde-activating, protein 5	-1.13	1.16	-3.76	4.36	3.55
pyrroloquinoline, quinone, biosynthesis, protein, PqqC	1.74	-1.28	1.04	-1.49	1.83
pyrroloquinoline, quinone, biosynthesis, protein, PqqE	1.65	-1.13	1.39	-1.72	1.28
pyrroloquinoline, quinone, biosynthesis, protein, PqqB	1.79	-1.21	-1.06	-1.26	2.23
aldehyde, dehydrogenase	1.22	-1.16	1.08	-1.32	1.19
aldehyde, oxidase	-1.37	-1.63	-1.66	-1.14	1.32
aldehyde, dehydrogenase 2	1.34	1.35	2.77	-1.87	-2.29

Comparisons: (A) AMS + CH4 vs. NMS + CH4; (B) NMS + MeOH vs. NMS + CH4; (C) AMS + MeOH vs. NMS + CH4; (D) AMS + MeOH vs. NMS + MeOH; (E) AMS + MeOH vs. AMS + CH4

Differential regulation of methane oxidation pathway genes in *M.* sp. Rockwell in either methane or methanol, with ammonium or nitrate. Columns A-E represent the fold-change differences in abundance of transcripts (RPKM) between different comparisons of four nutrient conditions. This pathway represents the central energy-generating pathway in these bacteria, as well as the source of fixed carbon.

RESULTS

Growth optimization in varying carbon/nitrogen/copper conditions



***Methylomicrobium album* BG8 grown in varying nutrient sources.** Optical density (540nm) of the cultures (n=3) grown in methane (top) and methanol (bottom) in nitrate and ammonia and in varying copper concentrations (1x Cu = 5μM).

FUTURE DIRECTIONS

- Analysis and mapping of the metabolome of strains of interest under different growth conditions.
- Transcriptomic analysis of four strains of methanotroph with regards to carbon and nitrogen source, to evaluate carbon flux and identify interaction effects of these two major nutrients.
- Transcriptomic analysis to understand effects of copper on growth and explore copper-based regulation in methanotrophs.

FES PROJECT OVERVIEW

T01-P03 Bioconversion of Single-Carbon Effluents into Biofuels and Biofuel Precursors

The aim of this project is to develop a platform technology for the bioconversion of C1 compounds resulting from forestry activities (fermentation, thermal processing, anaerobic digestion) into biofuels (alcohols, lipids) and biofuel precursors (e.g. isoprenoids). This platform will be integrated in the greater context of biomass conversion by, for example, using by-product streams from other bioconversion activities (e.g. anaerobic digestion and pyrolysis) as feedstock.

With thanks to our partners and funding agencies:

This research has been undertaken thanks in part to funding from the Canada First Research Excellence Fund.

[1]Department of Biological Sciences, University of Alberta, Edmonton, AB, Canada; [2]Department of Chemical and Materials Engineering, University of Alberta, Edmonton, AB, Canada