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Supplementary Materials for

Metabolic cross-feeding structures the assembly of polysaccharide degrading communities

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Other Supplementary Material for this manuscript includes the following:

Datasets S1 to S6

Supplementary datasets

Supplementary Dataset 1: LC-MS measurements of metabolites produced by degraders and exploiters after growth on chitin or GlcNAc

Supplementary Dataset 2: FIA-QTOF-MS measurements of metabolites produced by degraders and exploiters after growth on GlcNAc or chitin, measurements of metabolites produced by exploiters after growth on enzyme digest of degraders, and measurements of metabolites produced and consumed by all strains after growth on pooled supernatant

Supplementary Dataset 3: Compiled metabolite production and consumption data of each species that participated in 6 member chitin cocultures. This data was used for coculture specific metabolic network construction. The production and consumption data is compiled from individual metabolite production and consumption data of degraders grown on chitin, GlcNAc oligomers that degraders form extracellularly, secretion profiles of exploiters after growth in pooled supernatant, and metabolites consumed by exploiters and scavengers in pooled supernatant.

Supplementary Dataset 4: Hypothetical degrader-derived and exploiter-derived metabolite pools used for construction of hypothetical metabolic exchange networks and Boolean logic modelling to explain the outcome of six member chitin degrading cocultures.

Supplementary Dataset 5: 12 Hypothetical metabolic exchanged networks that were constructed based on the outcome of six member chitin degrading cocultures.

Supplementary Dataset 6: Proteins related to chitin degradation and metabolism found to be present in the species used in this work.

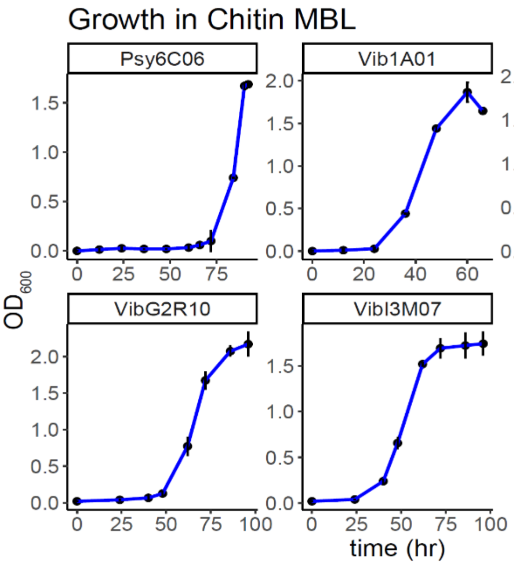

Fig S1: Growth of degraders on Chitin MBL containing 2g/l colloidal chitin. Error bars represent standard deviation of three biological replicates

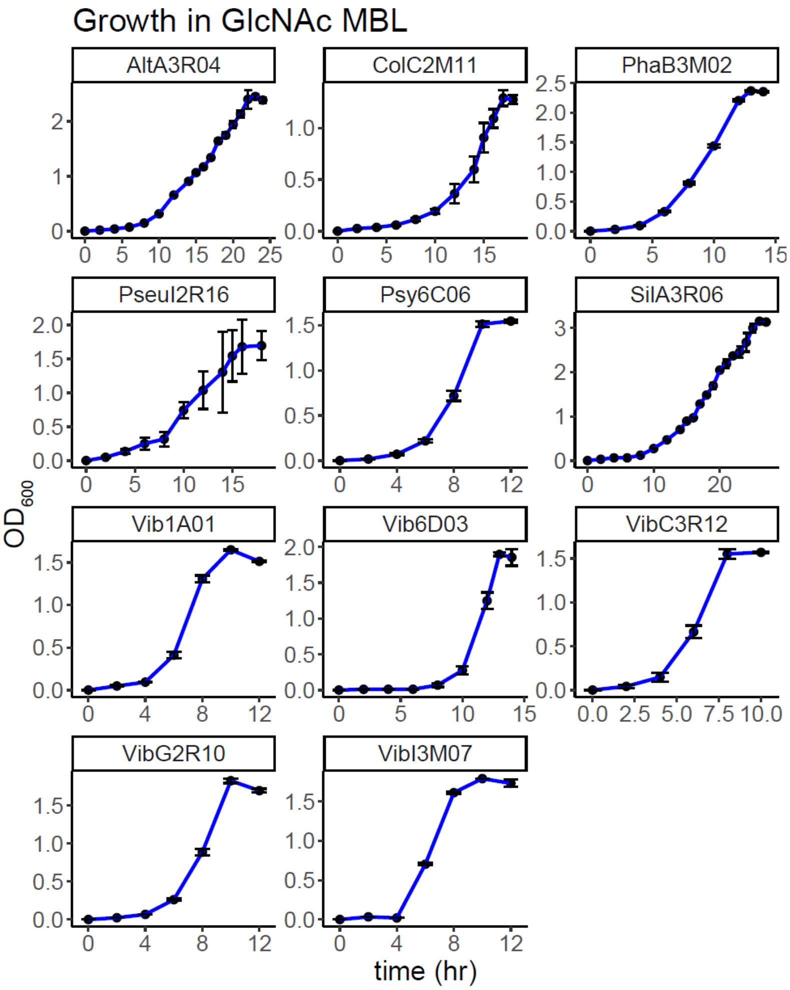

Fig S2: Growth of degraders and exploiters on GlcNAc MBL containing 20mM GlcNAc. Error bars represent standard deviation of three biological replicates

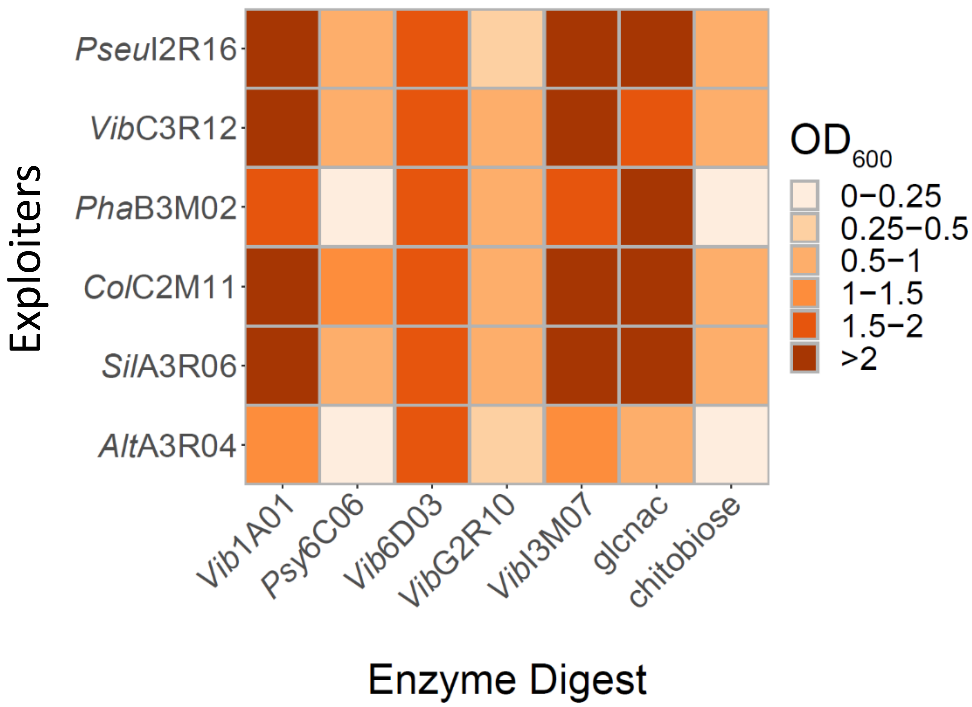

Fig S3: Growth of exploiters (vertical axis) after 36 h on colloidal chitin that was digested by enzymes concentrated from supernatants of the five degraders, 20mM GlcNAc or 10mM chitobiose (horizonal axis). OD_{600} data is normalized by the amount of enzyme collected from each degrader as this affects the amount of oligomer liberated from undigested chitin. These values are (left to right) 97.1, 251, 140, 39.3, and 93.6 µg/ml.

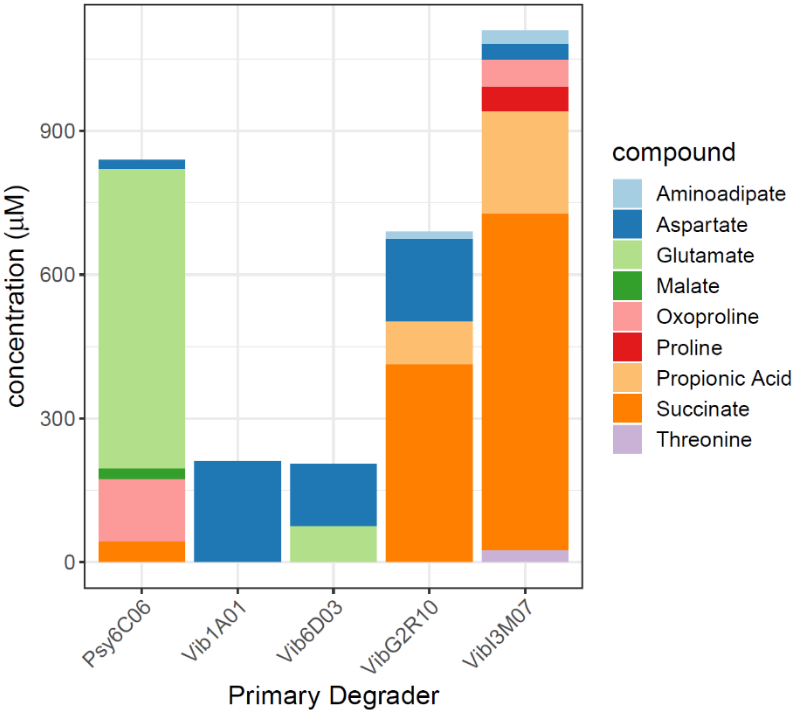

Fig S4: LC-MS measurements of metabolites secreted by degraders after growth on Chitin. Measurements represent the average of three independent biological replicates.

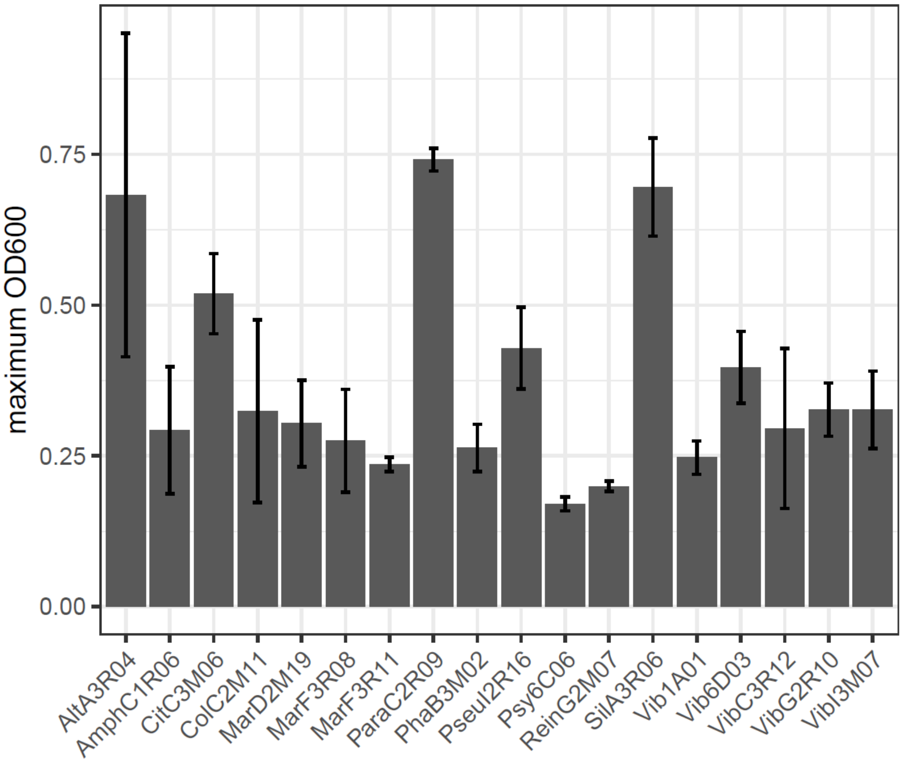

Fig S5: OD_{600} of each species after growth on pooled supernatant at 24 hours. Error bars represent standard deviation of three biological replicates.

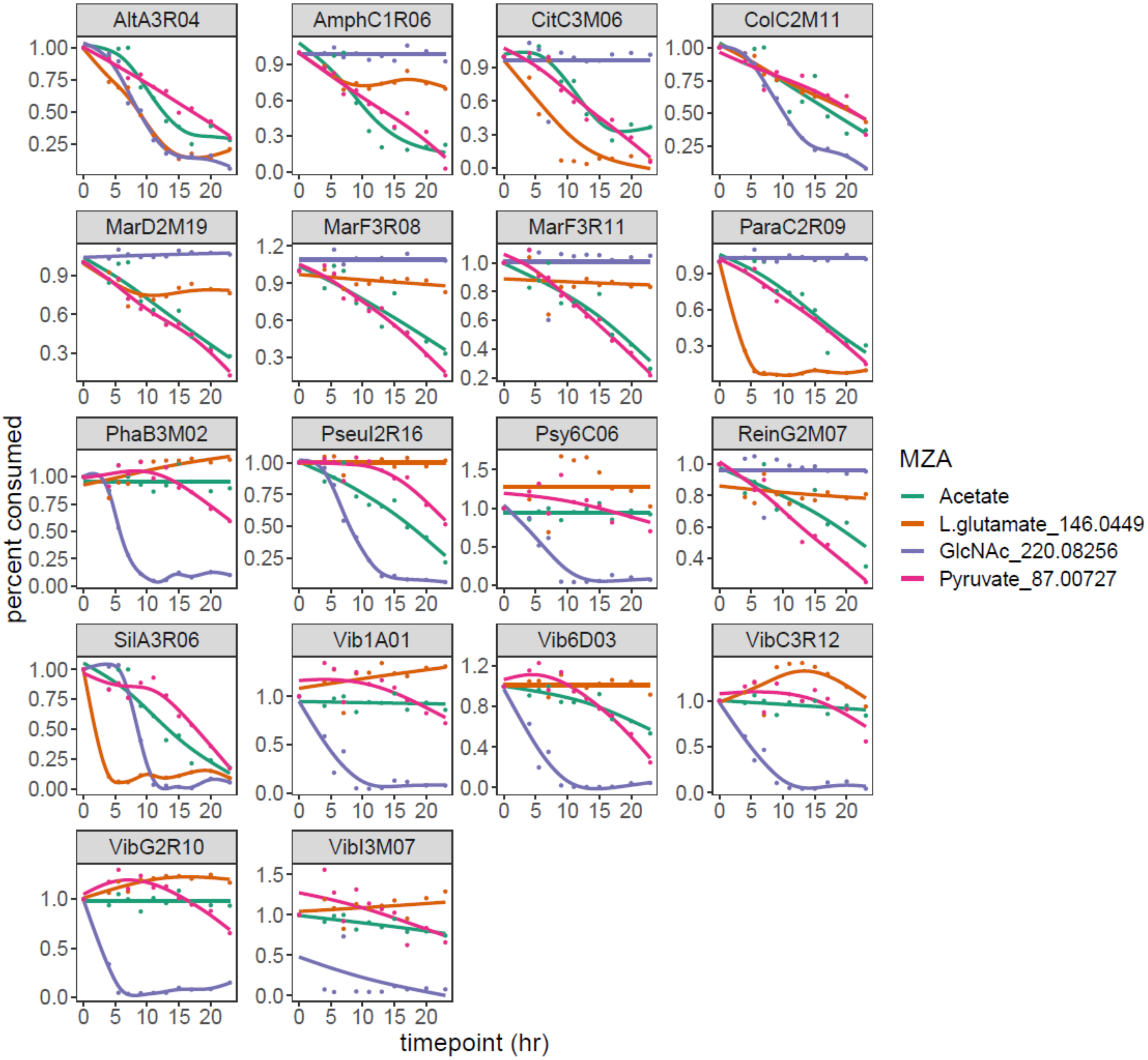

Fig S6: FIA-QTOF-MS timecourse measurements of consumption of four metabolites by each species when grown in pooled supernatant. Curves are fit using local polynomial regression.

Species	Taxonomic ID	Functional Guild	NCBI BioProject	NCBI BioSample
AltA3R04	Alteromonas sp.	Exploiter	PRJNA478695	SAMN19350919
AmphC1R06	Amphritea sp.	Scavenger	PRJNA478695	SAMN19350932
CitC3M06	Citricella sp.	Scavenger	PRJNA478695	SAMN19350936
ColC2M11	Colwellia psychrerythraea	Exploiter	PRJNA478695	SAMN19350933
MarD2M19	Marinobacter sp.	Scavenger	PRJNA478695	SAMN19350941
MarF3R08	Marinobacter sp.	Scavenger	PRJNA478695	SAMN19350954
MarF3R11	Marinobacter sp.	Scavenger	PRJNA478695	SAMN09522136
ParaC2R09	Paracoccus kamogawaensis	Scavenger	PRJNA478695	SAMN19350935
PhaB3M02	Phaeobacter sp.	Exploiter	PRJNA478695	SAMN09522130
PseuI2R16	f__Pseudoalteromonadaceae	Exploiter	PRJNA478695	SAMN19350960
Psy6C06	Psychromonas sp.	Degrader	PRJNA414740	SAMN08130274
ReinG2M07	Reinekea sp.	Scavenger	PRJNA478695	SAMN19350955
SilA3R06	Silicibacter sp.	Exploiter	PRJNA478695	SAMN19350920
Vib1A01	Vibrio splendidus	Degrader	PRJNA414740	SAMN07809270
Vib6D03	Vibrio penaeicida	Degrader	PRJNA414740	SAMN08130373
VibC3R12	Vibrio sp.	Exploiter	PRJNA478695	SAMN09522132
VibG2R10	Vibrio sp.	Degrader	PRJNA478695	SAMN19350956
VibI3M07	Vibrio sp.	Degrader	PRJNA478695	SAMN19350961

Table S1: Species used in this study and NCBI accession numbers for accessing genomic sequences.

	*Sil*A3R06	*Alt*A3R04	*Vib*C3R12	*Col*C2M11
*Vib*1A01	85%	23%	0%	0%
*Psy*6C06	83%	44%	0%	17%
*Vib*6D03	67%	33%	0%	33%

Table S2: Percentage of metabolites that are consumed by exploiters (top row) from the degrader-derived metabolite pools that otherwise have the capacity to be consumed by scavengers.

Coculture		Number of metabolites	
Degrader	Exploiter	Consumed	Secreted
1A01	A3R04	6	6
1A01	A3R06	19	0
1A01	C3R12	2	13
1A01	C2M11	4	10
6C06	A3R04	11	5
6C06	A3R06	21	0
6C06	C3R12	1	13
6C06	C2M11	5	10
6D03	A3R04	2	5
6D03	A3R06	7	0
6D03	C3R12	0	0
6D03	C2M11	3	10

Table S3. Number of metabolites consumed or secreted by exploiters in every exchange network.

Species	Metabolite	m/z
*Alt*A3R04	NA	
*Sil*A3R06	NA	
*Col*C2M11	NA	
*Vib*C3R12	Glutamate	146.0449
*Para*C2R09	Isocitrate	191.01952
	Alanine	88.03792
	Pantothenate	218.10352
	citraconate	129.0183
*Mar*D2M19	Isocitrate	191.01952
	2,3-dihydroxy-3-methylbutanoate	133.04882
	Malate	133.01218
	Ketovaline	115.03877
	Fumatate	115.00186
	Glycolate	75.00696
	Isobutanal	71.04872
*Cit*C3M06	Alanine	88.03792
	Pantothenate	218.10352
	Citraconate	129.0183
*Mar*F3R11	Isocitrate	191.01952
	Citraconate	129.0183

Table S4: Potential causal metabolites identified with Boolean logic that contribute to the growth of exploiters or scavengers in cocultures.

Primer target name	Primer sequence
1A01 F	GCACAGACCGTCCAAGAGAA
1A01 R	ACGGTCGCAAGCATATCAGT
C1R06 F	GCATTCGTCGTCAGCGTAAC
C1R06 R	GCAGGTTTTGCGTCGTAAGG
G2M07 F	GCGCTGCACTATAGCCTGTA
G2M07 R	CACGGGCTTTGAGGTAGTGT
G2R10 F	AGTGAAACCACCAATCGGCA
G2R10 R	GCACTATTGCTGTTAGGCGC
F3R08 F	AAGACTGCTACAGTGGCCAC
F3R08 R	CAGCAACGCCAGAAAAGTCC
6C06 F	AGCACACCCTCGCTCTAAAC
6C06 R	TGAGAAAGTCCTGTTCCGCC
A3R04 F	GCTTGCCACCTTTCCCAAAG
A3R04 R	TCCGGGCACATTGACAATCA
A3R06 F	GAGTTACCCCATGTTCCGCA
A3R06 R	AATTGTCATTGCCCGATGCG
C2M11 F	TGAAGCGACTTTTGCAGTGC
C2M11 R	CTGCAAGGGAGTAATGCGGA
B3M02 F	AAAGGCGCTCGTCTTTGGTA
B3M02 R	AGATGGGAAAGGTCATGCGG

Table S5: qPCR Primers used in this study