We currently have recovered the following methanogen MAGs/genomes from metagenomic sequencing of three different samples: (1) sequencing of DNA extracted from some cow rumen fluid samples that were used to set up cultures/enrichments in our lab (2) sequencing of DNA extracted from soil enrichments I performed using Old Woman Creek (our Ohio wetland) derived soil fed 13C-labeled substrates [acetate or dimethylsulfide] or (3) sequencing of DNA extracted from soil enrichments I performed using Old Woman Creek (our Ohio wetland) derived soil fed non isotopically-labeled substrates [various methyl compounds, acetate, hydrogen].

You can focus on two main things in the DRAM distillate: (1) the metabolism\_summary.xlsx and the product.html. The product is a heatmap showing either the percent completion of difference carbon metabolic pathways and respiratory complexes MAGs encode, or the presence/absence of specific metabolic marker genes. The metabolism summary shows you *all* the genes that DRAM v1.4 annotates/focuses on, and how many homologs your genomes/MAG encodes of each (0-n).

|  |  |
| --- | --- |
| **Rumen** | **GTDB Taxonomy** |
| **Su22\_1856\_I\_D90\_Am\_bin.41** | **d\_\_Archaea;p\_\_Thermoplasmatota;c\_\_Thermoplasmata;o\_\_Methanomassiliicoccales;f\_\_Methanomethylophilaceae;g\_\_UBA71;s\_\_** |
| **Su22\_1856\_I\_D90\_E\_bin.152** | **d\_\_Archaea;p\_\_Thermoplasmatota;c\_\_Thermoplasmata;o\_\_Methanomassiliicoccales;f\_\_Methanomethylophilaceae;g\_\_Methanarcanum;s\_\_** |
| **Su22\_1894\_T\_D90\_D\_bin.235** | **d\_\_Archaea;p\_\_Thermoplasmatota;c\_\_Thermoplasmata;o\_\_Methanomassiliicoccales;f\_\_Methanomethylophilaceae;g\_\_UBA71;s\_\_** |
| **Su22\_1894\_T\_D90\_A\_bin.224** | **d\_\_Archaea;p\_\_Thermoplasmatota;c\_\_Thermoplasmata;o\_\_Methanomassiliicoccales;f\_\_Methanomethylophilaceae;g\_\_Methanarcanum;s\_\_Methanarcanum sp017455785** |
| **Su22\_1894\_T\_D90\_A\_bin.232** | **d\_\_Archaea;p\_\_Thermoplasmatota;c\_\_Thermoplasmata;o\_\_Methanomassiliicoccales;f\_\_Methanomethylophilaceae;g\_\_UBA71;s\_\_** |
| **Su22\_1845\_C\_D90\_A\_bin.94** | **d\_\_Archaea;p\_\_Thermoplasmatota;c\_\_Thermoplasmata;o\_\_Methanomassiliicoccales;f\_\_Methanomethylophilaceae;g\_\_UBA71;s\_\_** |
| **Su22\_1894\_T\_D90\_A\_bin.77** | **d\_\_Archaea;p\_\_Thermoplasmatota;c\_\_Thermoplasmata;o\_\_Methanomassiliicoccales;f\_\_Methanomethylophilaceae;g\_\_UBA71;s\_\_** |
| **Su22\_1856\_I\_D90\_A\_bin.493** | **d\_\_Archaea;p\_\_Thermoplasmatota;c\_\_Thermoplasmata;o\_\_Methanomassiliicoccales;f\_\_Methanomethylophilaceae;g\_\_UBA71;s\_\_** |
| **Su22\_1856\_I\_D90\_Am\_bin.578** | **d\_\_Archaea;p\_\_Thermoplasmatota;c\_\_Thermoplasmata;o\_\_Methanomassiliicoccales;f\_\_Methanomethylophilaceae;g\_\_UBA71;s\_\_** |
| **OWC SIP Enrichment/Pilot - labeled** |  |
| **OWC\_0818\_M1\_M\_E017E020E021E022\_Coassembly\_bin.36** | **d\_\_Archaea;p\_\_Halobacteriota;c\_\_Methanosarcinia;o\_\_Methanosarcinales;f\_\_Methanosarcinaceae;g\_\_Methanomethylovorans;s\_\_Methanomethylovorans sp002508425** |
| **OWC\_0818\_M1\_M\_E022\_D31\_DNA\_S89\_L002\_bin.10** | **d\_\_Archaea;p\_\_Thermoplasmatota;c\_\_Thermoplasmata;o\_\_Methanomassiliicoccales;f\_\_UBA472;g\_\_MVRC01;s\_\_** |
| **OWC\_0818\_M1\_M\_E017E020E021E022\_Coassembly\_bin.50** | **d\_\_Archaea;p\_\_Thermoplasmatota;c\_\_Thermoplasmata;o\_\_Methanomassiliicoccales;f\_\_UBA472;g\_\_MVRC01;s\_\_** |
| **OWC\_0818\_M1\_M\_E017E020E021E022\_Coassembly\_bin.12** | **d\_\_Archaea;p\_\_Thermoplasmatota;c\_\_Thermoplasmata;o\_\_Methanomassiliicoccales;f\_\_UBA472;g\_\_;s\_\_** |
| **OWC\_0818\_M1\_M\_E022\_D31\_DNA\_S89\_L002\_bin.7** | **d\_\_Archaea;p\_\_Thermoplasmatota;c\_\_Thermoplasmata;o\_\_Methanomassiliicoccales;f\_\_UBA472;g\_\_MVRC01;s\_\_** |
| **OWC\_0818\_M1\_M\_E022\_D31\_DNA\_S89\_L002\_bin.8** | **d\_\_Archaea;p\_\_Halobacteriota;c\_\_Methanosarcinia;o\_\_Methanosarcinales;f\_\_Methanosarcinaceae;g\_\_Methanomethylovorans;s\_\_Methanomethylovorans sp002508425** |
| **OWC\_0818\_M1\_M\_E021\_D31\_DNA\_S88\_L002\_bin.7** | **d\_\_Archaea;p\_\_Halobacteriota;c\_\_Methanosarcinia;o\_\_Methanosarcinales;f\_\_Methanosarcinaceae;g\_\_Methanomethylovorans;s\_\_Methanomethylovorans sp002508425** |
| **OWC SIP Enrichment/Pilot - unlabeled** |  |
| **OWC\_0818\_M1\_M\_E05.bin.15** | **d\_\_Archaea;p\_\_Halobacteriota;c\_\_Methanosarcinia;o\_\_Methanosarcinales;f\_\_Methanosarcinaceae;g\_\_Methanomethylovorans;s\_\_Methanomethylovorans hollandica** |
| **OWC\_0818\_M1\_M\_CoA1\_E05E08E09\_bin.40** | **d\_\_Archaea;p\_\_Halobacteriota;c\_\_Methanosarcinia;o\_\_Methanosarcinales;f\_\_Methanosarcinaceae;g\_\_Methanosarcina;s\_\_** |
| **OWC\_0818\_M1\_M\_E09.bin.38** | **d\_\_Archaea;p\_\_Halobacteriota;c\_\_Methanosarcinia;o\_\_Methanosarcinales;f\_\_Methanosarcinaceae;g\_\_Methanomethylovorans;s\_\_Methanomethylovorans sp002508425** |
| **OWC\_0818\_M1\_M\_E09.bin.39** | **d\_\_Archaea;p\_\_Thermoplasmatota;c\_\_Thermoplasmata;o\_\_Methanomassiliicoccales;f\_\_Methanomethylophilaceae;g\_\_Methanogranum;s\_\_** |

We can have recovered a total of 20 medium and high-quality MAGs [https://www.nature.com/articles/nbt.3893 by Bowers et al 2017 ; see table 1]. We have two primary goals in working with these:

1. Specifically look at a subset of these MAGs to help us do a genome-guided methanogen isolation effort. For this, focus on the yellow highlighted MAGs
   1. For the rumen MAGs, those in yellow are MAGs recovered from metagenomic sequencing of rumen fluid sampled from Cow #1856 (look at the full MAG name!). We set up reactors using fluid from that exact cow, and thus have a match between sequence data, recovered methanogen genome, and culture in lab to try isolation from. We otherwise recovered methanogen MAGs from sequencing of rumen fluid sampled from Cows #1894 and 1845, but we do *not* have any reactors set up using fluid from those cows. So, our best aim is to use the 1856 sequencing data/genomes to guide isolation efforts of these specific methanogens from our enrichments established with fluid from cow 1856.
      1. FYI, Kelly’s dream is for us to isolate either ‘o\_\_Methanomassiliicoccales;f\_\_Methanomethylophilaceae’ or another member of that order.
   2. For the OWC enrichments, the unlabeled cultures were performed >1 year ago, and we do not have them anymore, so we can’t isolate anything from these
   3. For the OWC labeled enrichments, the distinct cultures were named E017, E018 . . . E022. We only sequenced cultures E017, E020, E021, E022.
      * 1. E017 was a soil enrichment that was fed hydrogen but no exogenous carbon
        2. E020 was a soil enrichment fed 13C2-acetate
        3. E021 was a soil enrichment fed 13C-dimethyl sulfide
        4. E022 was a soil enrichment fed 13C-dimethly sulfide *and* hydrogen
      1. I still have all of these cultures. However, if you look at the GTDB taxonomy of the MAGs we are most interested in, our goal is to pursue isolating a member of: ‘o\_\_Methanomassiliicoccales’
         1. Meaning, MAGs within the ‘o\_\_Methanosarcinales’ aren’t as interesting to us and we don’t need to pursue them for isolation efforts.
      2. I have highlighted three MAGs, all from culture E022, in yellow that we are interested in.
         1. We are not interested in the other MAGs that have the word “Coassembly” in the name
            1. This is because when you do a coassembly – we can talk more about this – you pool metagenomic sequence data from multiple samples to increase your chance of recovering MAGs (more sequence input = more likely you’ll have more reads from any single species that you can stitch together). *BUT*, this means a MAG that is from a coaasembly cannot be linked to one single culture for us to guse guided isolation efforts.
         2. SO, the three yellow “E022” MAG are what we can chase via isolation efforts, and we’ll use that specific single DMS +H2 fed culture as our inoculum source! So, we can analyze those genomes to understand what we should consider In designing dilution culture conditions.

**The goal then is to look at the specific methylotrophic gene content (tabs in the metabolism summary: METHYL, mtxB\_methyl\_curation\_15Jan24) to take a guess what methylated substrates these methanogens might utilize for grow, so that we can use them in methanogen isolation attempts (I’ve done this already for these MAGs, but lets see if we agree).**

* For this, focus on just the metabolism\_summary.xlsx
* Look at the METHYL tab (this includes a bunch of known methylotrophic genes, not all are related to methanogenesis). I’m included some supplemental files from my Stordalen Mire methylotrophy paper to help you figure out which genes matter most here – a figure with a visual table of known methanogenic methylotrophic genes and substrates should be enough. Table S2 from my paper also has two tables in the excel file with background info and references on methylotrophic genes, but this is likely more than you need here)
* *Things to look at for:*
  + Do these methanogens have *any* methylotrophic genes? (FYI, all the MAGs we have in this list happen to be known obligate methylotrophic methanogens, so they should)
  + What substrate categories do these genomes look to encode methyl genes for – methylamines? Methyl oxygen compounds (methanol, methoxy)? Methyl sulfides?
  + Are these specific substrates you can suggest that we could try using to isolate methanogens on? If there is redundancy/multiple options (i.e. one genome can use methyl N and O and S do you have any reasons to try some but not others?)

During the week I’m out, if you can, spend some time exploring these two DRAM outputs and start thinking about what physiological/metabolic capacities these methanogens seem to encode. Focus mostly on the carbon tabs, the energy tab, and the methyl tab (don’t really worry about the others for now).

Thing’s to look for:

* What methanogenesis genes do you see these MAGs enode?
* Do you see particular modules under the energy tab that these MAGs seem to encode all or most of?
* Are there particular carbon metabolic pathways that these MAGs seem to encode?
* Are there any transporters encoded that might seem logical based on the other gene content you see (i.e. if a MAG seems to encode methylamine use potential, and has a methylamine transporter?)

*Don’t worry about a formal write up or anything, but some notes (in excel/word/whatever you like) on what you did and what you saw in the data would be great!*

**Goal #2 if time allows (we’ll get to this eventually)**: Even though we aren’t trying to isolate all of the methanogens that we have genomes for in this list (n=20), these are new genomes for our internal methanogen genome database, so we might as well physiologically characterize them so we understand their metabolic potential!

**For this**, you can still focus on the metabolism summary, but the product.html can be useful too. Really, lets focus on catabolism and energy metabolism, so you can focus in the metabolism\_summary on the energy and carbon tabs (the MISC tab is mostly housekeeping related stuff, and the organic nitrogen is really amino acid metabolism, we don’t need to worry about these as much to start with).

Let’s say look at the product.html first – what general things do you notice in your MAGs? What carbon fixation pathways do they encode [FYI, to say a MAG actually encodes a pathway, lets for now set a threshold of encoding at least 50% of a pathway] Do your MAGs encode electron transport chain complexes? What other metabolic features does the product show your MAGs encode?

Looking at the metabolism\_summary.xlsx we can dig a bit more now, again just do carbon and energy tabes for now. Things to note

* Are there specific substrate-specific genes or modules you notice your MAGs encode potential to use? Do they encode genes for particular carbon substrates (i.e polyphenols)?
* Do your methanogen MAGs encode genes for alternative energy pathways beyond methanogenesis?
* Do your MAGs actually encode the pathway of methanogenesis? If so, which types/modules (acetoclastic, hydrogenotrophic, methloytrophic)?
  + Do your MAGs encode the wood-ljungdahl pathway? Acetate use genes? \
  + Do your MAGs encode an ATP synthase?
  + What hydrogenases do your MAGs encode?

*Just start playing around in and organizing the data if time allows, so we can start building an analysis of what these MAGs might actually have potential to do!*