

Supplementary material

Supplementary material for: Analysis of non-coding RNAs in *Methylobacterium extorquens* reveals a novel small RNA specific to Methylobacteriaceae

Emilie Boutet^a, Samia Djerroud^a, Katia Smail^a, Marie-Josée Lorain^b, Meiqun Wu^b,
Martin Lamarche^{a,b}, Roqaya Imane^a, Carlos Miguez^b and Jonathan Perreault^{*a}

^a INRS - Centre Armand-Frappier Santé Biotechnologie, 531 boulevard des Prairies,
Laval, QC, H7V 1B7, Canada,

^b National Research Council Canada, 6100 Royalmount, Montréal, Québec, Canada,
H4P-2R2.

Corresponding author: Jonathan.perreault@inrs.ca, 531 des Prairies Blvd, Laval, Quebec,
H7V 1B7, 450-687-4411

Analysis of non-coding RNAs in *Methylorubrum extorquens* reveals a novel small RNA specific to Methylobacteriaceae

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Tables

Table S1. Probes for candidates tested by Northern blot analysis

ID	Start	End	Size	Strand	Probe
426	51119	51301	182	+	GGCAGCCCAGCCTGCTGCTCCTTCAGGATGCCGATGATCTGCTCTTCGCT
432	80737	80895	158	+	CGGGCTCACGGTCTTTGGCCGTGAACCCTAAAAGTGCGGCGATATGAACC
609	636257	636362	105	+	GAAGGATCCTCCAGGGATCGCGCGGGATCTGGACGATCCTTCGTGGCCGC
1113	2218259	2218369	110	+	TGTGCTGATGTTTCCGCTTCTGCGGATCACCGTCACGAAAAACCCGAGCGG
1153	2388747	2388799	52	+	TCTGGAGCCCTCCTTCGAGGCCTCCGCTTCGCTCCGGCACCTCAGGATGA
1175	2436125	2436237	112	+	AGCTCGTCAGGCTCATAACCTGAAGGTCGCTGGTTCAAATCCAGCCCCCG
1348	3229332	3229428	96	+	AAGCTCGGCCGTATGTGAACCTTGGCCGTTCCGGCAGCGTTTCCCGAATGG
1627	4198109	4198315	206	+	CACGCGGGGAGAGGGTTCGCGACGACCACGG
1679	4343857	4343949	92	+	CGGAACTTGCAAACCATCTGCAAGCCGTAACCCCGACGCCGAAGGCATCA
1752	4567059	4567168	109	+	CGCGACGACCGGATGCGCCCTCGACGGGCGCTTCCGCCGCCGGCACGATC
1776	4678233	4678297	64	+	GCCTGCGAGCGCCCTGCCGGGCACTTCACGTGGGCCATCCGCCGGAACGG
1819	4874156	4874225	69	+	GTGGGATCGCGTTGGCCGCTATCCCGCGCT
1969	5367912	5368067	155	+	CGTCCGGTGTTCGTGGCGGGCCACTCAGTCCAAGACCGAACGGGTTCTCA
2036	51121	51275	154	-	AGACGGACGATGAAGAAGAGCCGGTTTAGCGAAGAGCAGATCATCGGCAT
2038	54339	54419	80	-	TCTGATCGTTGCCTCGCGTGCTAGACCCATGCGCGGGTCGCCGCAATGCG
2039	57158	57258	100	-	TAGCGCCGCCGAGGCGCGCGGGCAAGAGCCCGTTCCGGCCACGCCGGTA
2043	80757	80895	138	-	AATGCCTTCGGGCTCACGGTCTTTGGCCGTGAACCCTAAAAGTGCGGCGA
2185	636269	636362	93	-	AGCGGCCACGAAGGATCGTCCAGATCCCGCGCGATCCCTGGAGGATCCTTC
2624	2125529	2125634	105	-	AACGGCGCAGAGAAGTGGTCCGCACCGCCCGTCACTGCCGGCCCCGCCA
2674	2388747	2388799	52	-	CCTCATCCTGAGGTGCCGGAGCGAAGCGGAGGCCTCGAAGGAGGGCTCCA
3211	4753719	4753781	62	-	GACACCTTCAACTAGAAGGCGTCGCCGGACTCGATCCGGTGAGAACG GG
3355	5367928	5368087	159	-	TGAGAACCCGTTCCGGTCTTGACTGAGTGGCCCCGCCAACACCCGGACG

Table S2. Intergenic regions containing Met2624.

More conserved region containing Met2624 based on alignment results of all intergenic regions are italicized.

Hits from Genomic data
<p>><i>Methylobacterium extorquens</i> strain TK0001 ACAGGGCCTCGCATCAAAGCTCCCAGATCACCAGATCGCTGTCCCTCGGGGCACGGCCGCGTGGCGGGA GTTCTGTGAGAAGCAGGTCAGCCGAGGATCGGGAAGGGCCGGAACCCACATCGGTGGCAGGGGAGC GGCGTACGGCACGGTTGCCGTCCGGCGGCGGGGCCTCTACATCGGCCCTCCCCGCGGCCGATGCGC <u>CTGACGGTTTCGCGAGCCCTCTCGGGCCTGCGTGGTGGCGGGGCCGGCAGTGACGGGGCGGTGCGG</u> <u>ACCAGTTCTCTGCGCCGTTCTGTGACGCTCAGTGGGCCGAGCGGCTGCAGGTGCGTCGGGAGCGCG</u> <u>GGGAACAGGAACCGCGACCCCGGCGCAAGGCCACCTATCCGAATTCCCTCGACCGAATTCCTTAG</u> <u>CCCCTGGCCGACGCATCCGGTCCGGGGCCGGGCCTGGGATACAGCGCGAGAAGCGTTAACGGTC</u> <u>TTTCTCAAGGAAAGCGAACCAGGTAGCAGGCGATTTATCGCAGATTGTGCGCGAAAGGTGCGACCA</u></p> <p>><i>Methylobacterium zatmanii</i> strain PSBB041 ACAGGGCCTCGCATCAAAGCTCCCAGATCACCAGATCGCTGTCCCTCGGGGCACGGCCGCGTGGCGGGA GTTCTGTGAGAAGCAGGTCAGCCGAGGATCGGGAAGGGCCGGAACCCACATCGGTGGCAGGGGAGC GGCGTACGGCACGGTTGCCGTCCGGCGGCGGGGCCTCTACATCGGCCCTCCCCGCGGCCGATGCGC <u>CTGACGGTTTCGCGAGCCCTCTCGGGCCTGCGTGGTGGCGGGGCCGGCAGTGACGGGGCGGTGCGG</u> <u>ACCAGTTCTCTGCGCCGTTCTGTGACGCTCAGTGGGCCGAGCGGCTGCAGGTGCGTCGGGAGCGCG</u> <u>GGGAACAGGAACCGCGACCCCGGCGCAAGGCCACCTATCCGAATTCCCTCGACCGAATTCCTTAG</u> <u>CCCCTGGCCGACGCATCCGGTCCGGGGCCGGGCCTGGGATACAGCGCGAGAAGCGTTAACGGTC</u> <u>TTTCTCAAGGAAAGCGAACCAGGTAGCAGGCGATTTATCGCAGATTGTGCGCGAAAGGTGCGACCA</u></p> <p>><i>Methylobacterium extorquens</i> strain PSBB040 ACAGGGCCTCGCATCAAAGCTCCCAGATCACCAGATCGCTGTCCCTCGGGGCACGGCCGCGTGGCGGGA GTTCTGTGAGAAGCAGGTCAGCCGAGGATCGGGAAGGGCCGGAACCCACATCGGTGGCAGGGGAGC GGCGTACGGCACGGTTGCCGTCCGGCGGCGGGGCCTCTACATCGGCCCTCCCCGCGGCCGATGCGC <u>CTGACGGTTTCGCGAGCCCTCTCGGGCCTGCGTGGTGGCGGGGCCGGCAGTGACGGGGCGGTGCGG</u> <u>ACCAGTTCTCTGCGCCGTTCTGTGACGCTCAGTGGGCCGAGCGGCTGCAGGTGCGTCGGGAGCGCG</u> <u>GGGAACAGGAACCGCGACCCCGGCGCAAGGCCACCTATCCGAATTCCCTCGACCGAATTCCTTAG</u> <u>CCCCTGGCCGACGCATCCGGTCCGGGGCCGGGCCTGGGATACAGCGCGAGAAGCGTTAACGGTC</u> <u>TTTCTCAAGGAAAGCGAACCAGGTGGCAGGCGATTTATCGCAGATTGTGCGCGAAAGGTAGCACCA</u></p> <p>><i>Methylobacterium</i> sp. AMS5 GGCGGTCTTCGGGGCAAAGCGTCGCCCCAGGGCAGCGGCGTACGGCACGGTTGCCGTCCGGCGG CGCAGGCCTCTACATCGGCCCTCCCCGCGGCCGATGCGCCCGATGGTTTCGCGAGCCCTCTCGGGCC <u>TGCGTGGTGGCGGGGCCGGCAGTGACGGGGCGGTGCGGACCAGTTCTCTGCGCCGTTCTGTGACGC</u> <u>TCAGTGGGCCGAGCGGCTGCAGGTGCGTCCGGGAGCGCGGGGAACAGGAACCGCGACCCCGGCGCAA</u> <u>GGCCACCTACC</u><u>TGAATTCCTCGCCCAATTCCTTGGCCCCAGACCGGCACGCGTCCGGTCCGGG</u> <u>GCCGGCCTGGGATATCGGCGAGCGAAGCGTTAACGGTCTTTCTCAAGGAAAGCGAACCAGCTCGT</u> <u>GGCGATCTATCGCAGACCATCGCTAAAGGTGCGACCA</u></p> <p>><i>Methylobacterium extorquens</i> strain DM4 ACACGGCCTCGCATCAAAGCTCCCAGATCACCAGATCGCTGTCCCTCGGGGCACGGCCGCGTGGCGGGA GTTCTGTGAGAAGCAGGTCAGCCGAGGGCCGCGCAAGGCTGGAACGTACATCGGTGGCGGGGGAGC GGCGTACGGCACGGTTGCCGTCCGGCGGCGGGGCCTCTACATCGGCCCTCCCCGCGGCCGATGCGC <u>CTGACGGTTTCGCGAGCCCTCTCGGGCCTGCGTGGTGGCGGGGCCGGCAGTGACGGGGCGGTGCGG</u> <u>ACCAGTTCTCTGCGCCGTTCTGTGACGCTCAGTGGGCCGAGCGGCTGCAGGTGCGTCGGGAGCGCG</u> <u>GGGAACAGGAACCGCGACCCCGGCGCAAGGCCACCTATCCGAATTCCCTCGACCGAATTCCTTAG</u> <u>CCCCAGACCGGCACGCATCCGGTCCGGGGCCGGGCCTGGGTACAGCGCGAGAAGCGTTAACGGTCT</u> <u>TTTCTCAAGGAAAGCGAACCAGGTGGCAGGCGATTTATCGCAGATTGTGCGCGAAAGGTGCGACCA</u></p> <p>><i>Methylobacterium extorquens</i> AM1 ACAGGGCCTCGCATCAAAGCTCCCAGATCACCAGATCGCTGTCCCTCGGGGCACGGCCAGGTGGCGGG AGTTCTGTGAGAAGCAGGTCAGCCGAGGGCCGCGCAAGGCCGGAACCCACATCGGTGGCGGGGGAG CGGCGTACGGCACGGTTGCCGTCCGGCGGCGGGGCCTCTACATCGGCCCTCCCCGCGGCCGATGCG <u>CCTGACGGTTTCGCGAGCCCTCTCGGGCCTGCGTGGTGGCGGGGCCGGCAGTGACGGGGCGGTGCGG</u> <u>GACCAGTTCTCTGCGCCGTTCTGTGACGCTCAGTGGGCCGAGCGGCTGCAGGTGCGTCGGGAGCGC</u> <u>GGGAACAGGAACCGCGACCCCGGCGCAAGGCCACCTATCCGAATTCCCTCGACCGAATTCCTTAG</u> <u>GCCCCAGACCGGCACGCATCCGGTCCGGGGCCGGGCCTGGGATACAGCGCGAGAAGCGTTAACGGT</u> <u>CTTTCTCAAGGAAAGCGAACCAGGTGGCAGGCGATTTATCGCATATTGTGCGCGAAAGGTGCGACCA</u></p> <p>><i>Methylobacterium extorquens</i> CM4 ACAGGGCCTCACAAAGGCTCCCAGATCACCAGATCGCTGTCCCTCGGGGCACGGCCGCGTGGCGGG AGTTCTGTGAGAAGCAGGTCACCCGAGGACCGGCGAGGGCCAGACCCACGTGCGGCGGGGGAG CGGCGTACGGCACGGTTGCCGTCCGGCGGCGGGGCCTCTACATCGGCCCTCCCCGCGGCCGATGCG <u>CCTGACGGTTTCGCGAGCCCTCTCGGGCCTGCGTGGTGGCGGGGCCGGCAGTGACGGGGCGGTGCGG</u> <u>GACCAGTTCTCTGCGCCGTTCTGTGACGCTCAGTGGGCCGAGCGGCTGCAGGTGCGTCGGGAGCGC</u> <u>GGGAACAGGAACCGCGACCCCGGCGCAAGGCCACCTATCCGAATTCCCTCGACCGAATTCCTTAG</u> <u>GCCCCAGACCGGCACGCATCCGGTCCGGGGCCGGGCCTGGGATACAGCGCGAGAAGCGTTAACGGT</u> <u>CTTTCTCAAGGAAAGCGAACCAGGTGGCAGGCGATTTATCGCATATTGTGCGCGAAAGGTGCGACCA</u></p>

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ATGCATCCGGTCCGGGGCCGGGCTGGGATACAGCGCGAGAAGCGTTAACGGTCTTCTCAAGGGA
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>*Methylobacterium* sp. N191

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>*Methylobacterium* sp. CLZ

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>*Methylobacterium* sp. YC-XJ1

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>*Methylobacterium* sp. DM1

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>*Methylobacterium* sp. DM1

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>*Methylobacterium* sp. DM1

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>*Methylobacterium* sp. DM1

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>*Methylobacterium* sp. DM1

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>*Methylobacterium* sp. DM1

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> *Methylobacterium mesophilicum* SR1.6/6

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> *Methylobacterium* sp. C1

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> *Methylobacterium radiotolerans* JCM 2831

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> *Methylobacterium* sp. XJLW

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> *Methylobacterium phyllosphaerae* strain CBMB27

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> *Methylobacterium oryzae* CBMB20

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> *Methylobacterium radiodurans* strain 17Sr1-43

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>*Methylobacterium* sp. WL1

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>*Methylobacterium nodulans* ORS 2060

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>*Methylobacterium currus* strain PR1016A

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>*Methylobacterium* sp. 17Sr1-1

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>*Methylobacterium indicum* VL1 DNA

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>*Methylobacterium aquaticum* strain BG2

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>*Methylobacterium aquaticum* strain MA-22A

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Supplementary material

Hits from Metagenomic data

>PJQF01118147.1

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> FUFK010045813.1

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Table S3. Intergenic regions containing Met1153.

More conserved region containing Met1153 based on alignment results of all intergenic regions are italicized.

Hits from Genomic data
<p>>Methylobacterium extorquens AM1 ATCGCCTGTTCCGGCGAATGGGGTCAGCCGGTTGGTGGATCAGATTTCGTTCTCCGGCCAATCCCCTCA TCCTGAGGTGCCGGAGCGAAGCGGAGGCCTCGAAGGAGGGCTCCAGAAGACGCTGCGATTCTTGGA GCCCTCCTTCGAGGCCGCTCCGCGCACCTCAGGATGAGGTAAGTCTGGGAAAACAGTCGAATAG AATCTCAAGCGAACGGCAGCATCAGCGGCAGCGCGAGCAGGGCGCCGGCGGCCAGGACGATGGCAT <u>CGCGCAGGGCGCCGGCCGATCCAGGGCGGGCGTCCGGGATGCTGTGCGTGGGTGTCTTCGAAAAACGT</u> <u>CATGGTCTGTCTCCTGTCCGTTTCGATGGCCCCGATATTACGGCGCCCGCCTTTCCCGACAGGGACGG</u> TTGCGGTACGATTTCAACGAACTGTCCGGGACGAGCACCAGATACAGTTCGGAGGGACAGCCA</p> <p>>Methylobacterium zatmanii strain PSBB041 CATGGAGCGGGCGAAGGGCTGTTTCGAGGCGAACTGCACCGCCGGTGTATCGCTGTTCCGGCGAATG GGGTCAGCCGGTTGGTGGATCAGATTTCGCTCCTCCGGCCAATCCCCTCATCTGAGGTGCCGGAGCGA AGCGGAGGCCTGGAAGGAGGGCTCCAGAAGACGCTGCGATTCTGGAGCCCTCCTTCGAGGCCGCTC CGCGGCACCTCAGGATGAGGTAAGTCTGGGAAAACAGTCGAATAGAATCTCAGGGCGAACGGCAC <u>GATCAGCGGCAGCGGAGCAGGGCGCCGGCGGAGGACGATGGCATCGCGCAGGGCGCCGGCCGAT</u> <u>CCAGCGCGGGGATCGGGATGCTGTGCGTGGGTGCTTCAAAAAACGTATGGTCTGTCTCCTGTCCGG</u> <u>TTCGATGGCCCCGATATGACGGCGCCGCGCTTTCCCGACAGGGACGGTTGCGGTACGATTTCAACGA</u> ACTGTCCGGGACGAGCACCAGTACAGTTCGGAGGGACAGCCATGACGGTGACGGTTCGCGGAACCGGT CCTCTCGCGGGTCGAGACGGTGATG</p> <p>>Methylobacterium extorquens strain TK 0001 GAGCGAAGCGGAGGCCTGGAAGGAGGGCTCCAGAAGACGCTGCGATTCTGGAGCCCTCCTTCGAGG CCGTCCGCGGCACCTCAGGATGAGGTAAGTCTGGGAAAACAGTCGAATAGAATCTCAGGGCGAAC <u>GGCAGCATCAGCGGCAGCGGAGCAGGGCGCCGGCGGAGGACGATGGCATCGCGCAGGGCGCCGG</u> <u>CCGATCCAGGGCGCGGATCGGGATGCTGTGCGTGGGTGCTTCAAAAAACGTATGGTCTGTCTCCT</u> <u>GTCGGTTCGATGGCCCCGATATGACGGCGCCGCGCTTTCCCGACAGGGACGGTTGCGGTACGATTTCA</u> AACGAACTGTCCGGGACGAGCACCAGTACAGTTCGGAGTGACAGCCATGACGGTGACGGTTCGCGGA ACCGGTCTCTCGCGGGTCGAGACGGTGATGCGGGCCATCGAGGCGCGGATCGAGGGCCGGGCGCTC GGCCCCGGCGCGCGGTGCCCTCGGTGCGGAGCCTCGCCGACA</p> <p>>METHYLORUBRUM EXTORQUENS STRAIN PSBB040 TGGTCCCCCTCCGAACGTACCGATGCTCGTCCCGGACAGTTCGTTGAAATCGTACCGCAACCGTCC CTGTCCGGAAAGCGGCGCCGTATATCGGGCCATCGAACCAGACAGGAGACAGACCATGACGTT <u>TTTCGAAGACACCCACGCCAGCATCCCCAGCCTCGCGCTGGATCGGTCCGGCGCCTGCGCGATGCC</u> <u>ATCGTCTCCGCGCCGGCGCCCTGCTCGCGTCCCGCTGATCGTGCCGTTCCGCTGAGATTCTATTCCG</u> <u>ACTGGTTTTCCAGGACTTACCTCATCCTGAGATGCCGCGGAGCGGCCTCGAAGGAGGGCTCCAGGA</u> ATCGCAGCGTCTTCTGGAGCCCTCCTTCGAGGCCTCCGTTTCGCTCCGGCACCTCAGGATGAGGGGAT TGGCCGGAGGAGCGAATCTGATCCACCAACCGGCTGACCCATTTCGCCGAACACGCGATCAGCCGGC GGTGCAAGTTCGCTCGAACAGCGCCTTCGCCCTCCTATGGCCCGGAACCGCCGACCGGAGCCCGGG CTGCGCAGTTCGCGCCCTCGGGCAGACGCCCCAGGAAGGGTTCGGACAGGTCCGCCACGAACGGCC GGGGCCCGGTATAGGCGCCATGCGGTTGCGCAGATCGAACCCTGCCGACAAGGCCCGCGCCCGGCC GATCCGACGGCCGGCCACCTTCGCCTCGGGTTCGCGCATCTGCCGCTCGATCAGGCTCAGCGCGGTCC GCGCCGCTCCGCCCGACGCCGGCTCCGAACCCTCACGACGGTCTGAGCGCCCGCCGACCCCGC GCCGGCAGGATCAGGAAGGCCCGGAGGGCGGTCTTGCGCAGGCGCAT</p> <p>>Methylobacterium extorquens CM4 GAGGCGAAGTGCACCGCCGGTGTATCGCTGTTCCGGCGAATGGGGTCAGCCGGTTGGTGGATCAGAT TCGTCTCCTCCGGCCAATCCCCTCATCTGAGGTGCCGGAGCGAAGCGGAGGCCTCGAAGGAGGGCTC CAGAAGACGCTGCGATTCTCGGAGCCCTCCTTCGAGGCCGCTCCGCGGCACCTCAGGATGAGGTAAG TCCTGGGAAAACAGTCGAATAGAATCTCAGGGCGAACGGCAGCATCAGCGGTAGCGCGAGCAGGGC <u>GCCGGCGCCAGGACGATGGCATCGCGCAGGGCGCCGGCCGATCCAGGGCGGGGACCAGGATGCTG</u> <u>TGCTTGGGTGTCTTCAAAAAACGTATGGTCTGTCTCCTGTCCGTTTCGATGGCCCCGATATGACGGCG</u> <u>CCGCGCTTTCCCGACAGGGACGGTTGCGGTACGATTTCAACGAACTGTCCGGGACGAGCGCTGGTA</u> CAGTTCGGAGAGACAGTACGAGTTCGCGGAACCGGT</p> <p>>Methylobacterium extorquens str. DM4 chromosome GAGGCGAAGTGCACCGCCGGTGTATCGCTGTTCCGGCGAATGGGGTCAGCCGGTTGGTGGATCAGAT TCGTCTCCTCCGGCCAATCCCCTCATCTGAGGTGCCGGAGCGAAGCGGAGACCTCGAAGGAGGGCTC CAGAAGACGCTGCGATCTCTGGAGCCCTCCTTCGAGGCCGCTCCGCGGCACCTTAGGATGAGGTAAG TCCTGGGAAAACCCAGTCGAAGAGCCGCTCAGGGCGAACGGCAGCATCAGCGGTAGCGCGAGCAGGGC <u>GCCGGCGCCAGGACGATGGCATCGCGCAGGGCGCCGGCCGATCCAGGGCGGGGACCAGGATGCTG</u> <u>TGCTTGGGTGTCTTCAAAAAACGTATGGTCTGTCTCCTGTCCGTTTCGATGGCCCCGATATGACGGCG</u> <u>CCGCGCTTTCCCGACAGGGACGGTTGCGGTACGATTTCAACGAACTGTCCGGGACGAGCGCTGGTA</u> CAGTTCGGAGAGACAGTACGAGTTCGCGGAACCGGT</p> <p>>Methylobacterium extorquens str. DM4 chromosome GAGGCGAAGTGCACCGCCGGTGTATCGCTGTTCCGGCGAATGGGGTCAGCCGGTTGGTGGATCAGAT TCGTCTCCTCCGGCCAATCCCCTCATCTGAGGTGCCGGAGCGAAGCGGAGACCTCGAAGGAGGGCTC CAGAAGACGCTGCGATCTCTGGAGCCCTCCTTCGAGGCCGCTCCGCGGCACCTTAGGATGAGGTAAG TCCTGGGAAAACCCAGTCGAAGAGCCGCTCAGGGCGAACGGCAGCATCAGCGGCAGCGCGAGCAGG <u>GCGCCAGCGCGAGGACGATGGCATCGCGCAGGGCGCCGGCCGATCCAGGGCGGGGCGTCCGGGATGC</u> <u>TGTGCGTGGGTGTCTTCAAAAAACGTATGGTCTGTCTCCTGTCCGTTTCGATGGCCCCGATATGACGG</u> <u>CGCCGCGCTTTCCCGACAGGGACGGTTGCGGTACGATTTCAACGAACTGTCCGGGACAAGCGTCCG</u> TACAGTTCGGAGGGACATCCATGACGGTTCGCGGAACCGGT</p>

Supplementary material

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>Methylobacterium populi BJ001

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>Methylorubrum populi strain YC-XJ1

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>Methylobacterium sp. CLZ

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>Methylobacterium sp. DM1

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>Methylorubrum sp. B1-46

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>Methylobacterium populi DNA

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Table S4. RNAcode results for intergenic region containing Met2624.

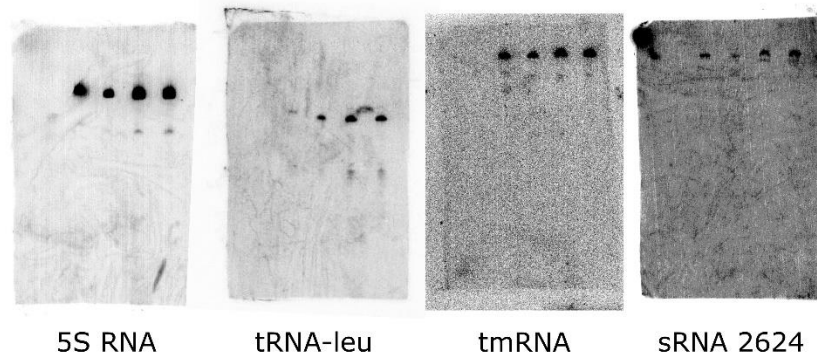
Frame	Amino acids (aa)			Nucleotide		Score	P
	Length	From	to	Start	End		
-2	29	3	31	8	94	17.62	0.305
+2	20	1	20	2	61	16.03	0.439
-3	9	54	62	162	188	11.46	0.890
+3	16	34	49	102	149	10.18	0.959
-1	9	34	42	100	126	10.05	0.964
+1	8	21	28	61	84	7.94	0.998
+2	4	23	26	69	80	7.14	1
-2	14	39	52	116	157	7.10	1
+3	3	5	7	15	23	6.45	1
+2	14	47	60	140	181	6.21	1
-1	3	5	7	15	23	5.71	1

Table S5: Optical density at which samples were taken for RNA-seq

Condition		Optical density (600 nm)
	1	3.206
Wild type, controlled pH	2	3.12
	3	3.125
	1	2.968
Triple mutant, controlled pH	2	2.902
	3	2.921
	1	2.958
Triple mutant	2	3.266
	3	3.207

Figures

A



B

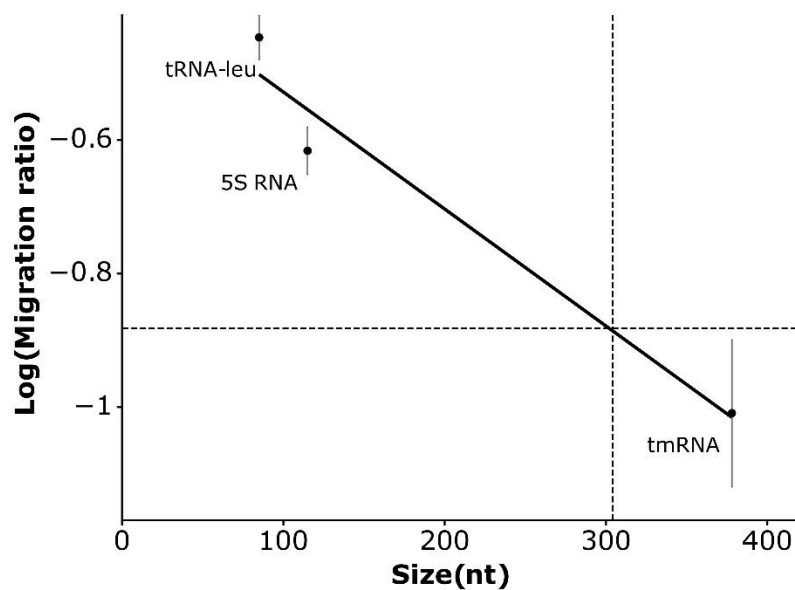


Figure S1. Size estimation of candidate sRNA2624 based on known RNA. Hybridization of probes for controlled RNA is observed with radioactivity labelling. Since the size of these controlled RNAs are known (5S RNA, 115 nt; tRNA-leu, 85 nt and tmRNA; 378 nt), their migration ratio in a membrane can be measured and graphed to estimate the size of candidate RNAs such as sRNA2624 (B). All probes were tested on three different membranes.

Supplementary material

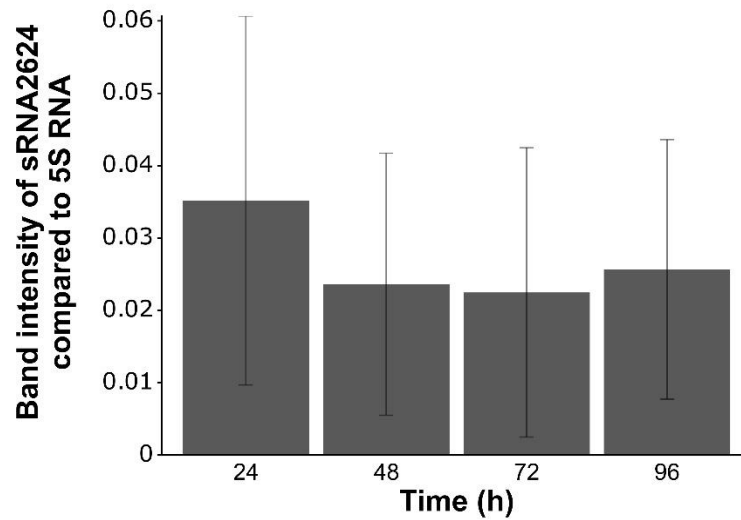


Figure S2. Expression of sRNA2624 over the growth of *M. extorquens*. Probes for sRNA 2624 were tested on three different membrane and normalized to the band intensity of a control RNA, 5S RNA.

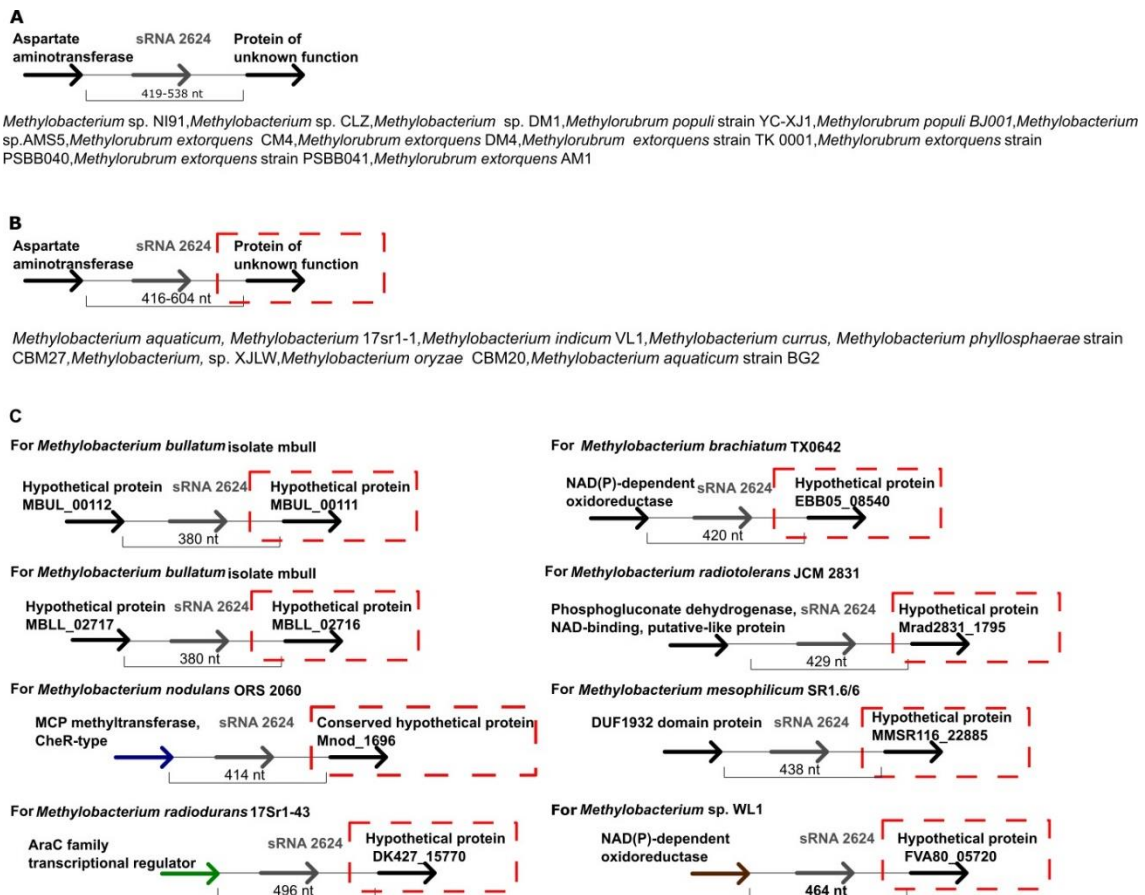


Figure S3. Genomic context of Met2624. Results are based on NCBI BLASTn [37]. All bacteria listed in (A) have an aspartate aminotransferase in 5' of Met2624 and a protein of unknown function of the same family in 3'. Bacteria listed in (B) also have an aspartate aminotransferase upstream of the sRNA candidate, but it is followed by a different protein

Supplementary material

of unknown function. Bacteria listed in (C) have the same protein of unknown function encoded downstream of Met2624 in their genome as those listed in (B) represented by a dashed red box, but the protein upstream differs. Proteins that are identical are represented by the same color in (C) (black, blue, brown, or green). Met2624 is encoded only in the family Methylobacteriaceae.

```
Sequences: 34
Columns: 200
Reading direction: forward
Mean pairwise identity: 83.71
Shannon entropy: 0.37902
G+C content: 0.72183
Mean single sequence MFE: -111.29
Consensus MFE: -73.21
Energy contribution: -68.23
Covariance contribution: -4.98
Combinations/Pair: 1.71
Mean z-score: -2.03
Structure conservation index: 0.66
Background model: dinucleotide
Decision model: sequence based alignment quality
SVM decision value: 1.47
SVM RNA-class probability: 0.941197
Prediction: RNA
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Figure S4. RNAz results for intergenic regions containing Met2624. The program suggested Met2624 is a functional RNA.

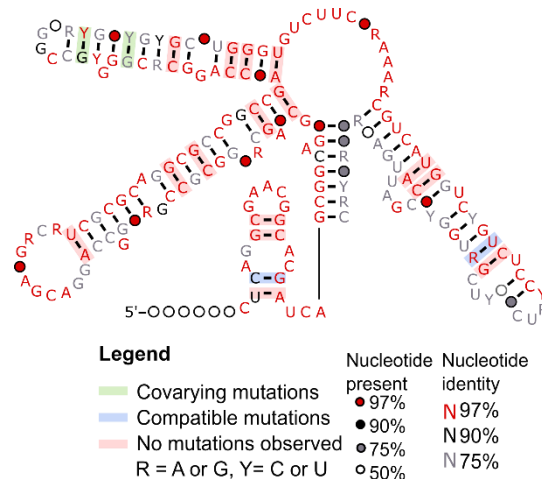


Figure S5. Secondary structure for Met1153. The structure was drawn by the program R2R [53]. Taken individually, none of the indicated covarying base pairs are considered statistically significant according to R-scape [44].

Supplementary material

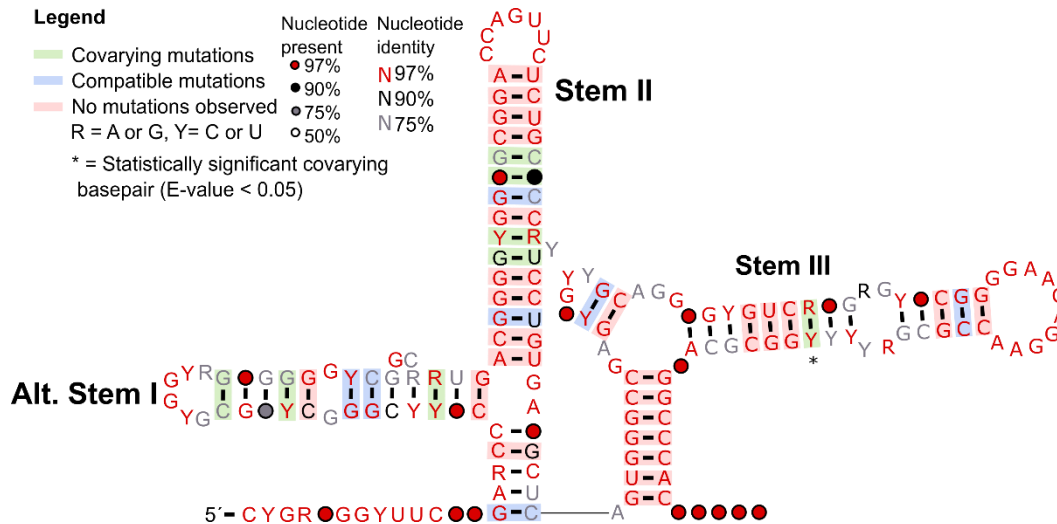


Figure S6. Alternative secondary structure for Met2624. The structure was drawn by the program R2R using an alternative Stockholm alignment [53]. Taken individually, a base pair in stem III is considered statistically significant according to R-scape [44]. While the structure pictured in Figure 5 fits the conserved region and the experimentally estimated 5' and 3' ends, several alternative alignments (and corresponding structure predictions) with slightly different 5' and 3' ends were performed (data not shown). For several of these structures as well as for the structure pictured in Figure S6, it can be noted that stems II and III are identical to that of Figure 5. This contrasts with the “Alternative stem I” which is completely different from Stem I in Figure 5 and also appears less likely to form, given that the latter has more conserved basepairs, covariations and compatible mutations.

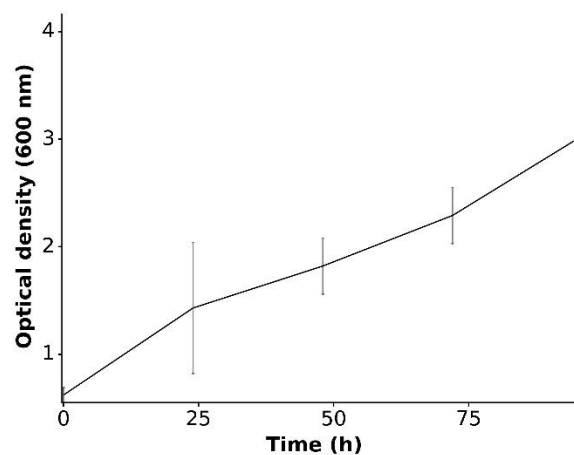


Figure S7. Growth curve of *M. extorquens* ATCC55366 for Northern Blot Analysis. It was cultivated with 1% methanol as the sole source of carbon in tri-replicates. The optical density (600 nm) was measured every 24 hours for four consecutive days.