Figure S1. Denaturing and non-denaturing PAGE of purified CmtA. Lanes 1 and 2, denaturing PAGE of molecular mass standards (kDa) and 5 µg CmtA stained with Coomassie G-250. Lane 3, non-denaturing gradient polyacrylamide gel of 10 µg CmtA stained for methylcob(III)alamin:CoM methyltransferase activity.

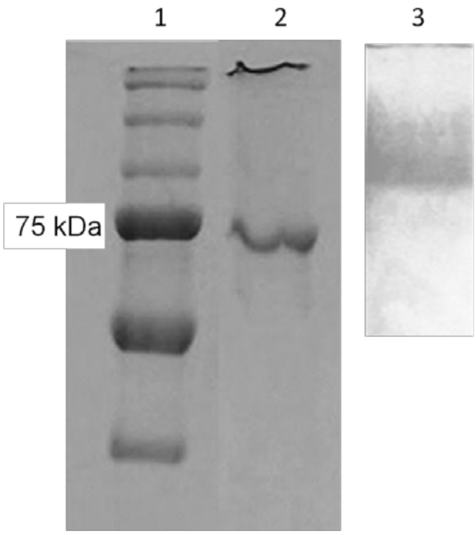

Figure S2. Comparison of sequences retrieved from a BLASP search queried with the deduced sequence of *M. acetivorans* MA4384. The retrieved sequences from *Methanohalobium evestigatum* (GENE ID: 9347354), *Methanohalophilus mahii* (GENE ID: 8983654) and *Methanosalsum zhilinae* (GENE ID: 10823438) are shown in panels A, B and C respectively. M.a. in each panel indicates the deduced sequence of MA4384 (GENE ID: 1476278).

Panel A.

```
M.a.  2     AKEDILNTLADAVVDGDDELAEEFAQKALDEE--LDAYEAIVDGLARGMKIISDMYERGE  59
            +KEDILN L +AV+ GD + A+E A K ++    +D YEAIVDGLA+GM I+SD YE GE
M.e.  6     SKEDILNGLTNAVIQGDQDSAKELATKVIENGGVVDPYEAIVDGLAKGMSIMSDKYENGE  65

M.a.  60    AFVPSLLLAADAMYAGMDILTPYMKVDGTAAPKNVIIGTVEGDVHDIGKNLVKTMMTAAG  119
             FVP LL+A++AMYAGM  LTPY+K D  +    VIIGTV+GDVHDIGKNLVKTM+TA+G
M.e.  66    VFVPHLLVASNAMYAGMKELTPYIKTDEASKQAIVIIGTVQGDVHDIGKNLVKTMLTASG  125

M.a.  120   FNMIDLGCDVPLDKFAETAKEKKAAAISMSTLMTTTMGGMETVIEQLQEEGIRDSLIVMV  179
            FN IDLG DV LD+F E AKE KA AISMS LMTTTMGGMETVI++L+EEG+RDS+IVMV
M.e.  126   FNAIDLGNDVQLDEFVEKAKENKADAISMSALMTTTMGGMETVIDKLKEEGLRDSVIVMV  185

M.a.  180   GGAPISQTFADSVGADGTALDASAAVETLTSLVSEL-PSDS-WSDSAIAASKMKYKEVLA  237
            GGAP+S+ +A+ +GAD T  DA +A + L   V EL P++  WS+  I+ SK+KY+E LA
M.e.  186   GGAPVSEDYANEIGADATLPDADSAADWLKDAVKELEPAERRWSEEKISTSKVKYREELA  245

M.a.  238   QKSGKEKVDIGRITAEKIIAEFDSVVPKFNETMTKAERFGAAFQDKKVDRLPVAPLACGV  297
            +K+  E+VDIGR TA++II E +SV  K  E MT  ER  ++  DKKVDRLPV PLACGV
M.e.  246   KKTVSEEVDIGRETAKEIIDEVESVGTKGKEEMTSIERVTSSLADKKVDRLPVYPLACGV  305

M.a.  298   SRKFVPCSYVDYSTKAEKYADCVEAGIKYFNMDTFVGLTDLCVDAADFGATIRYPEEDTP  357
            +RKFVP +Y  Y+   E +A    AG KY + D FVGL DL + A D G  I YPEEDTP
M.e.  306   TRKFVPTTYKQYAIDPEMFAQSAYAGAKYLDYDMFVGLIDLSITAGDLGCEITYPEEDTP  365

M.a.  358   AATGHLEDYEKLEVPELKEGTRAYNLIQGNKLATEKAHALDAPMTALIEGPMVALTQIMG  417
            ++ GHLEDYE++EVPE+KEGTRAY L+Q  KLA EK H +  P+    EGP++ LTQ+MG
M.e.  366   SSKGHLEDYEEIEVPEVKEGTRAYELVQATKLAKEKLHEIGKPVVGFHEGPLLTLTQLMG  425

M.a.  418   ATRVLSDLRTNPDVVLKALDKTTVYVEEIMKGMFEEAQPDNLCMVNLWTNNVILSADEYM  477
            A RV+ D++TNP+VV  A+DK   YV  + +  FEE   D LC+ NLW+NN I+S D+Y
M.e.  426   ADRVMMDMKTNPEVVRDAVDKCADYVNLVTEKFFEEDACDALCIDNLWSNNKIMSEDDYW  485

M.a.  478   KSEGQVMQNRIAPLYKKYNKPVVIHNCADAPHWEL-INKWNTEYYSYTFYPDEAGKGSKD  536
            K +G+ + ++  PL+KKY++P +IH+CAD+ H++  I+K+ T+ +SY +YP+E  +GSK+
M.e.  486   KFDGKFIVDKHVPLFKKYDQPYMIHSCADSVHYDTQISKFGTDLFSYAYYPNEREQGSKN  545

M.a.  537   HKYLINNYGKETMFGGEVSPIVFLDNSPEGLQKMKADTIALQESVLNTLKENGMQSKYMI  596
            +  LI  YG E    GEV PI F+DNS E + K+K+DT  +    LNTL ENG+QSKY++
M.e.  546   YSDLIPKYGHECCMMGEVDPIQFMDNSSETIDKIKSDTDNVLTGALNTLNENGLQSKYVV  605

M.a.  597   STGCEVPPGAPCDSITAQTYTVAEKGPELYKKIIG  631
            STGCE+PPG     +      V EKGP L KK +G
M.e.  606   STGCEIPPGGSLTGVKEMIDLVKEKGPGLQKKTMG  640
```

Panel B.

```
M.a.  4     EDILNTLADAVVDGDDELAEEFAQKALDEELDAYEAIVDGLARGMKIISDMYERGEAFVP  63
            +DIL  LA+AVV G+ +L  E ++KAL E LD ++AI+DGLA+GM I+SD YE+G AFVP
M.m.  3     QDILKELAEAVVSGNKDLTAELSEKALKEGLDPHKAIIDGLAKGMVIVSDNYEKGTAFVP  62

M.a.  64    SLLLAADAMYAGMDILTPYMKVDGTAAPKNVIIGTVEGDVHDIGKNLVKTMMTAAGFNMI  123
             LL+A+ AMYAGMD+LTP+++ + ++ P  ++IGTVEGDVHDIGKNLVKTMM+A G + I
M.m.  63    HLLIASQAMYAGMDVLTPHIETEESSKPATMVIGTVEGDVHDIGKNLVKTMMSAGGIDTI  122

M.a.  124   DLGCDVPLDKFAETAKEKKAAAISMSTLMTTTMGGMETVIEQLQEEGIRDSLIVMVGGAP  183
            DLG DVPLDKF ETA+E +A  ISMS LMTTTM GME VIE LQEEG+RDSLIVMVGGAP
M.m.  123   DLGNDVPLDKFIETARENQADVISMSALMTTTMSGMEKVIEMLQEEGLRDSLIVMVGGAP  182

M.a.  184   ISQTFADSVGADGTALDASAAVETLTSLVSEL-PS-DSWSDSAIAASKMKYKEVLAQKSG  241
            IS+ FA+++GAD TA DA  A       VS+L PS + WSD  I  +K+KY+E+LA+K
M.m.  183   ISEDFAENIGADKTAPDALHASNWAIDAVSKLSPSKERWSDEKINLAKVKYREILAKKQV  242

M.a.  242   KEKVDIGRITAEKIIAEFDSVVPKFNETMTKAERFGAAFQDKKVDRLPVAPLACGVSRKF  301
            K K DIGR TA++I+ EF+SV  K  E M+ A+R  AA  DKKVDRLPV PLACG  RKF
M.m.  243   KSKKDIGRETAKQIMEEFESVGVKSKEEMSHADRTLAAMADKKVDRLPVYPLACGALRKF  302

M.a.  302   VPCSYVDYSTKAEKYADCVEAGIKYFNMDTFVGLTDLCVDAADFGATIRYPEEDTPAATG  361
               +Y +Y+    K+A+    G KY ++D FVGL DL   +ADFG TI+YPEEDTP++ G
M.m.  303   ADATYKEYAIDPNKFAESAFLGCKYMDLDMFVGLIDLSATSADFGCTIKYPEEDTPSSEG  362

M.a.  362   HLEDYEKLEVPELKEGTRAYNLIQGNKLATEKAHA-LDAPMTALIEGPMVALTQIMGATR  420
            HLEDYE +E PE+KEGTRAY LI+ +KLA +K +  L  P     EGP++ LTQ+MGA R
M.m.  363   HLEDYEDIETPEVKEGTRAYELIEASKLAKDKLNKELGTPFVGFHEGPLLTLTQLMGADR  422

M.a.  421   VLSDLRTNPDVVLKALDKTTVYVEEIMKGMFEEAQPDNLCMVNLWTNNVILSADEYMKSE  480
            VL D++TNPDVVL+AL K T YV ++ +  FEE   D LC+ NLW+NN+I+  ++Y K +
M.m.  423   VLMDMKTNPDVVLEALQKCTDYVCQVSEAFFEEDACDALCVDNLWSNNIIMDEEDYWKFD  482

M.a.  481   GQVMQNRIAPLYKKYNKPVVIHNCADAPHWEL-INKWNTEYYSYTFYPDEAGKGSKDHKY  539
            G+ + ++  P++KKY++P +IHNCADA H++  I K+ T  +SY +Y  E  KGS+++
M.m.  483   GKFVYDQHIPVFKKYDQPYIIHNCADAVHFDTQIKKFGTALFSYAYYESEREKGSQNYAD  542

M.a.  540   LINNYGKETMFGGEVSPIVFLDNSPEGLQKMKADTIALQESVLNTLKENGMQSKYMISTG  599
            LI  YG      GE++PI  +DNSPEG+QK++ DT  L + V  +LKENGMQSKY++STG
M.m.  543   LIPKYGDMCCMMGEINPIDMMDNSPEGIQKIENDTEVLLKGVHESLKENGMQSKYVMSTG  602

M.a.  600   CEVPPGAPCDSITAQTYTVAEKGPELYKKIIG  631
            CE PPG P   I A    V E GP+  K IIG
M.m.  603   CETPPGGPMTPIKAMVDKVKELGPQFQKDIIG  634
```

Panel C.

```
M.a.  3    KEDILNTLADAVVDGDDELAEEFAQKALDEELDAYEAIVDGLARGMKIISDMYERGEAFV  62
           KE+ILN LADAVV G  E A E ++KAL+E +D  EA+V+GLA+GM I+SD YE GEAFV
M.z.  2    KEEILNQLADAVVSGSKESAAELSKKALEEGIDPQEALVNGLAKGMNIMSDKYETGEAFV  61

M.a.  63   PSLLLAADAMYAGMDILTPYMKVDGTAAPKNVIIGTVEGDVHDIGKNLVKTMMTAAGFNM  122
           P LL+A+ AMYAGM++L P++K +G   P   +IGT+EGDVHDIGKNLVKTM++AAGFNM
M.z.  62   PHLLIASGAMYAGMEVLIPHLKKEGAGRPATGVIGTIEGDVHDIGKNLVKTMLSAAGFNM  121

M.a.  123  IDLGCDVPLDKFAETAKEKKAAAISMSTLMTTTMGGMETVIEQLQEEGIRDSLIVMVGGA  182
           IDLG DVPL+ F + AKE KA  ISMS LMTTTM GME VIE LQEEGIRD++ VMVGGA
M.z.  122  IDLGNDVPLEDFVKAAKENKADIISMSALMTTTMVGMEKVIESLQEEGIRDAVKVMVGGA  181

M.a.  183  PISQTFADSVGADGTALDASAAVETLTSLVSELP--SDSWSDSAIAASKMKYKEVL--AQ  238
           P+S+ FA+ +GAD T  D+  A +  T+ V  +P   + W++  I+  K+KY++VL   +
M.z.  182  PVSEEFAEKIGADSTHPDSMGAADWATNAVKSMPPADERWTEEKISLGKVKYRDVLAKKK  241

M.a.  239  KSGKEKVDIGRITAEKIIAEFDSVVPKFNETMTKAERFGAAFQDKKVDRLPVAPLACGVS  298
                  DIG  TA +II EF+ V  K  E MT  +R  ++  DKKVDRLPV PLACGV
M.z.  242  AKVGAAKDIGLETAREIIDEFEKVGVKKKEEMTHIDRVVSSLGDKKVDRLPVYPLACGVL  301

M.a.  299  RKFVPCSYVDYSTKAEKYADCVEAGIKYFNMDTFVGLTDLCVDAADFGATIRYPEEDTPA  358
           RKFVP +Y +Y+T  E +A     G+KY ++D FVGL DL + ++D G  +  P+EDTPA
M.z.  302  RKFVPTTYREYTTDPEMFAQSAYLGVKYMDLDMFVGLVDLSITSSDLGCKVSIPDEDTPA  361

M.a.  359  ATGHLEDYEKLEVPELKEGTRAYNLIQGNKLATEKAHA-LDAPMTALIEGPMVALTQIMG  417
           + GHL+DY+K+E+PE+KEGTR Y LIQ +KLA E+ +  L+AP  A  EGP++ LTQ+MG
M.z.  362  SVGHLDDYDKIEIPEVKEGTRIYELIQASKLAKERLNKELNAPFVAFHEGPLLTLTQLMG  421

M.a.  418  ATRVLSDLRTNPDVVLKALDKTTVYVEEIMKGMFEEAQPDNLCMVNLWTNNVILSADEYM  477
           A RVL D++TNPDVVL A+DK T +V +I +  FEE   D LC+ NLW+NNVI+S D+Y
M.z.  422  ADRVLMDMKTNPDVVLDAVDKMTDFVCDISEKFFEEDACDGLCIDNLWSNNVIMSEDDYW  481

M.a.  478  KSEGQVMQNRIAPLYKKYNKPVVIHNCADAPHWEL-INKWNTEYYSYTFYPDEAGKGSKD  536
           K EG+ +++R  PL+K+YN+P +IH+CAD  H+++ I ++ T  +SY +YP    KGSK+
M.z.  482  KFEGKFVKDRHVPLFKEYNQPYMIHSCADNVHFDIQIKEFGTGLFSYAYYPKLRNKGSKN  541

M.a.  537  HKYLINNYGKETMFGGEVSPIVFLDNSPEGLQKMKADTIALQESVLNTLKENGMQSKYMI  596
           +  LI  YG      GEV PI F+D SPEG+Q ++ DT  L   VL  LKENG+QSKY++
M.z.  542  YADLIPKYGDMCCMMGEVDPIKFIDPSPEGVQSIENDTEELLRGVLPVLKENGLQSKYVM  601

M.a.  597  STGCEVPPGAPCDSITAQTYTVAEKGPELYKKIIG  631
           STGCE+PPG P + + A   TV + GP+L K+I+G
M.z.  602  STGCEIPPGGPLNGVKAMVDTVKKLGPDLQKEIMG  636
```

Figure S3. Comparison of the deduced sequence of MA4384 from *M. acetivorans* with sequences of subunits from the DMS:HS-CoM methyltransferase of *M. barkeri*. Panel A, MA4384 (GENE ID: 1476278) *versus* MtaA (GENE ID: 73671018); Panel B, MA4384 versus MtaB (GENE ID: 73671018).

Panel A.

```
MtsA     5 MTPTRRVMAAVLGGRVDYVPPANPLAQTTTELMQICNASWPKAHFDSKMMADLAAAPYEV  64
           MT   R  AA    +VD +P A PLA   +     C  S+      ++  AD   A  +
MA4384 270 MTKAERFGAAFQDKKVDRLPVA-PLACGVSRKFVPC--SYVDYSTKAEKYADCVEAGIKY  326

MtsA    65 CGIEAARPQFDISLEAEVLGCKLDWDKPDRPPVIGPAYTDPSNVTWPDNLEEAGRIPVVL  124
             ++      D+ ++A   G  + + + D P   G    D   +  P+ L+E  R   ++
MA4384 327 FNMDTFVGLTDLCVDAADFGATIRYPEEDTPAATGH-LEDYEKLEVPE-LKEGTRAYNLI  384

MtsA   125 GAIEELRTRYDGML--PVIPLLTSPFTIASHIAGVENMAKWTKTAPEKAHAFIEAATDFV  182
               +L T     L  P+  L+  P    + I G   +    +T P+     ++  T +V
MA4384 385 QG-NKLATEKAHALDAPMTALIEGPMVALTQIMGATRVLSDLRTNPDVVLKALDKTTVYV  443

MtsA   183 --IAYGKLQ----------TIYGAHIVFLADPSASGDLISAETYREFVLPAHKRIAKEIS  230
             I  G  +           ++  +++  AD     +    +  +  + P +K+  K
MA4384 444 EEIMKGMFEEAQPDNLCMVNLWTNNVILSADEYMKSE---GQVMQNRIAPLYKKYNK---  497

                * *
MtsA   231 CPQILHICGDTSKLLPYIKQSGIDCFSFDAVP---------VSYCRQVVGNEMSILGSLD  281
            P ++H C D       I +   + +S+   P           Y     G E    G +
MA4384 498 -PVVIHNCADAPH-WELINKWNTEYYSYTFYPDEAGKGSKDHKYLINNYGKETMFGGEVS  555

                                                       *
MtsA   282 VIDLMPN-------------GTPEQVYNRTRECILQGADIVGTSCGLSFGTSLENLRAYV  328
            I  + N                E V N  +E  +Q   ++ T C +  G   +++ A
MA4384 556 PIVFLDNSPEGLQKMKADTIALQESVLNTLKENGMQSKYMISTGCEVPPGAPCDSITAQT  615

MtsA   329 RACKE  333
               E
MA4384 616 YTVAE  620
```

Panel B.

```
MtsB    66  IAEAVITGNRAETVKLTTALLAEGRDPTDLVLNALMPGIHTVCELYDIGESYVPEILLAN  125
            +A+AV+ G+     +     L E  D  + +++ L  G+  + ++Y+ GE++VP +LLA
MA4384  10  LADAVVDGDDELAEEFAQKALDEELDAYEAIVDGLARGMKIISDMYERGEAFVPSLLLAA  69

MtsB   126  EALIGGVGLCQE--RIGEVPYQGKVVSLVIEGDVHDIGKNIVVAILRANGFEVVDMGSDV  183
            +A+  G+ +     ++        V+   +EGDVHDIGKN+V  ++ A GF ++D+G DV
MA4384  70  DAMYAGMDILTPYMKVDGTAAPKNVIIGTVEGDVHDIGKNLVKTMMTAAGFNMIDLGCDV  129

MtsB   184  TVEEAVKAVKATNADLVTGTTLMSTTKGGIKDLAEILYSEDVP----VACGGAAIDRNFV  239
             +++  +  K   A  ++ +TLM+TT GG++ + E L  E +     V  GGA I + F
MA4384 130  PLDKFAETAKEKKAAAISMSTLMTTTMGGMETVIEQLQEEGIRDSLIVMVGGAPISQTFA  189

MtsB   240  ET  241
            ++
MA4384 190  DS  191
```