LEGANDS FOR SUPPLEMENTARY FIGURES

FIG. S1. **Alignment of protein sequences of GOGAT β subunit-like proteins and β-subunits from *E. histolytica* and other organisms.** The sequences were aligned using the CLUSTAL W program. Accession numbers of these sequences are as follows: *Escherichia coli* (P09832), *Azospirillum brasilense* (AAG38999), *Clostridium saccharobutylicum* (AAP06761), *E. histolytica* EhNO1 and 2 (XP_656997 and XP_653573). The cysteine residues that are proposed to participate in the formation of [4Fe-4S] clusters are highlighted in bold. The residues that match the consensus sequence implicated in the formation of an adenylate binding fold in the FAD- or NAD(P)H-binding region are marked with a plus ("+"). A sharp ("#") indicates tyrosine residues that are proposed to be replaced by acidic residues in NADH-dependent enzymes, and a triangle ("▲") indicates the basic residue that is proposed to favour NADPH binding. Residues that match the second FAD consensus sequence are marked with an asterisk (*). The GXGXX(G/A/P) motif described in the text is shown in bold.

FIG. S2. **Phylogenetic reconstructions of GOGAT β subunit and GOGAT β subunit-like proteins from a variety of organisms.** Phylogenetic trees of 32 β-subunit and β-subunit-like proteins were constructed using the CLUSTAL W program and drawn with the MEGA4.1 program, and a representative neighbor-joining tree is shown. The number at the nodes represents the bootstrap value as a percentage of 1000 replicates. The scale bar indicates 0.1 substitutions at each amino acid position. Species names and accession numbers of the sequences are also indicated. The β subunit domains of the *M. sativa*, *A. thaliana*, *S. cerevisiae*, *P. falciparum*, *D. melanogaster*, and *C. elegans* polypeptides were included from residue positions 1676, 1701, 1635, 2513, 1625, and 1706, respectively.

FIG. S3. **Expression and purification of recombinant EhNOs.** Protein samples were electrophoresed on a 12% SDS-PAGE gel under reducing conditions, and then stained with Coomassie Brilliant Blue R250. Lane 1, molecular mass markers; lanes 2 and 3, total lysate of uninduced and isopropyl β-d-thiogalactopyranoside-induced *E. coli* cultures, respectively; lane 4, purified rEhNO1; lane 5, purified rEhNO2 (10 μg).

Suppl. Figure S1

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EhNO1                          MKSFNITHVLKRNPEERINDWKEVVVGYSKEETIEEASRCLGCKKPGCVPTC 52
EhNO2                         MAANYNRTPVPMRNPEERINDWKEVVVGYTKEDTKTEASRCLGCKKPGCVPTC 53
C. saccharobutylicum                                  MTCNDIQLLDEADRCLLCKKPRCKENC 27
E. coli                 MSQNVYQFIDLQRVDPPKKPLKIRKIEFVEIYEPFSEGQAKAQADRCLSCGNPYCEWKC 59
A. brasilense          MANQRMLGFVHTAQRMPDKRPAAERRQDFAEIYARFSDERANEQANRCSQCGVPFCQVHC 60


EhNO1                  PAHINIPEYIIHLKKGEFNEATNVILKDMPLLHICGRVCP--HYCEKTCVKAK-RGGELQ 109
EhNO2                  PAHIDIPGFISELKKEQYTEAVSVILKRLPFPNICGRVCP--HYCQGKCVKAR-RGGELE 110
C. saccharobutylicum   PIQTSIPEIISLYKENRLREAGEILFNNNPLSAVCSLVCIHEDQCKGNCIKGI-KGEPIR 86
E. coli                PVHNYIPNWLKLANEGRIFEAAELSHQTNTLPEVCGRVCPQDRLCEGSCTLND-EFGAVT 118
A. brasilense          PVSNNIPDWLKLTSEGRLEEAYEVSQATNNFPEICGRICPQDRLCEGNCVIEQSTHGAVT 120

                                                               FAD-I
EhNO1                  IMELKRSAITYANEEDIKIDIAP--DTSKKVAVVGSGPAGLAAAYFLRLKGHKVVVYEQK 167
EhNO2                  IMELKRSAITYSTEEDIKIDIAP--DTGKKVAVVGSGPAGLAAAYFLRLKGHKVVVYEQK 168
C. saccharobutylicum   FHDIEYEISMKYLEEVRFENLPK---DKDRIAIIGGGPAGITIAFILANKGYDVTIFDAH 143
E. coli                IGNIERYINDKAFEMGWRPDMSGVKQTGKKVAIIGAGPAGLACADVLTRNGVKAVVFDRH 178
A. brasilense          IGSVEKYINDTAWDQGWVKPRTPSRELGLSVGVIGAGPAGLAAAEELRAKGYEVHVYDRY 180
                                                    ++ + + +  +  +   +      + + +

EhNO1                  HKLGGMMILCIPPYRLPRDKLDEDIDRIKRLGIEFRTNAKVD---NIPSLLNE-YDAVFV 223
EhNO2                  HKLGGMMILCIPPYRLPRDKLDEDIDRIKRLGIEFRTNAKVD---DITTLKKE-YDAVFV 224
C. saccharobutylicum   VKIGGVLRYGIPEYRLPKKILDTIEDRLVELGIKIRPNTLIGPVITLDRLYEDGYKAIFI 203
E. coli                PEIGGLLTFGIPAFKLEKEVMTRRREIFTGMGIEFKLNTEVGRDVQLDDLLSD-YDAVFL 237
A. brasilense          DRMGGLLVYGIPGFKLEKSVVERRVKLLADAGVIYHPNFEVGRDASLPELRRK-HVAVLV 239


EhNO1                  GIGTLKRKVLGIPGEDLIGVEHVIPYLESINTFARK------------TIGKKVAVVGAG 271
EhNO2                  GIGTLKRKVLGIPGEDLIGVEHVIPYLESINTFARK------------TIGKKVAVVGAG 272
C. saccharobutylicum   GTGVWNPKTLNIKGETRGNAHFAIDYLKSPEAYRLG---------------EKVAVIGAG 248
E. coli                GVGTYQSMRGGLENEDADGVYAALPFLIANTKQLMG-FGETRDEPFVSMEGKRVVVLGGG 296
A. brasilense          ATGVYKARDIKAPGSGLGNIVAALDYLTTSNKVSLGDTVEAYENGSLNAAGKHVVVLGGG 299
                                                                           ++ + + +
                             NAD(P)H
EhNO1                  FSAMDAVRVARRLGS-EAFIIYRRSEHEMPASSSEIEEAKEEGVTMMTLCNPTKIIGDEN 330
EhNO2                  FSAMDAVRVARRLGS-EAFIVYRRSENEMPAAPSEVEEAKEEGVTMMTLCNPTKIIGDEN 331
C. saccharobutylicum   NVAMDAARSAKRNGAKEVTVLYRKGFDEMSATKQEIMEAKEDGVIFNLFKSPIEITEKGI 308
E. coli                DTAMDCVRTSVRQGAKHVTCAYRRDEENMPGSRREVKNAREEGVEFKFNVQPLGIEVNGN 356
A. brasilense          DTAMDCVRTAIRQGATSVKCLYRRDRKNMPGSQREVAHAEEEGVEFIWQAAPEG--FTGD 357
                         +   +  +       +   #▲

EhNO1                  GKVKGIECIKMKLGESDESGRAAPVPIPGSEFVIDCDMVIQAISQVVD----LGCCPGVE 386
EhNO2                  GKVKGIECIKMKLGEPDASGRAAPVPIPGSEFVIDCDMVIQAISQGVE----TECCKDVE 387
C. saccharobutylicum   R-------LSSTENVTDENGKIVTKVIEGKEEFFECDSIIIAVSQTPKTN-IVSNTKELN 360
E. coli                GKVSGVKMVRTEMGEPDAKGRRRAEIVAGSEHIVPADAVIMAFGFRPHNMEWLAKHS-VE 415
A. brasilense          TVVTGVRAVRIHLGVADATGRQTPQVIEGSEFTVQADLVIKALGFEPEDLPNAFDEPELK 417

                                           FAD-II
EhNO1                  LTQYGTLKVDENYK----TSINGLYAAGDCITGPKSIVDAVGDAHRASNAIHEYLTNLTI 442
EhNO2                  LSKWQTLQVNDKFQ----TNVEGIYASGDCVTGPKSIVHAVGDTYVAVEAMHEYLMGKKN 443
C. saccharobutylicum   TNKWGLIITDEKGN----TTRKGTFASGDVVTGAKTVVEAVVQAKTVANTIEEYCKNN   414
E. coli                LDSQGRIIAPEGSDNAFQTSNPKIFAGGDIVRGSDLVVTAIAEGRKAADGIMNWLEV    472
A. brasilense          VTRWGTLLVDHRTK---MTNMDGVFAAGDIVRGASLVVWAIRDGRDAAEGIHAYAKAKAE 474
                                         *        **

EhNO1                  TPMEQD   448
EhNO2                  EE       445
A. brasilense          APVAVAAE 482
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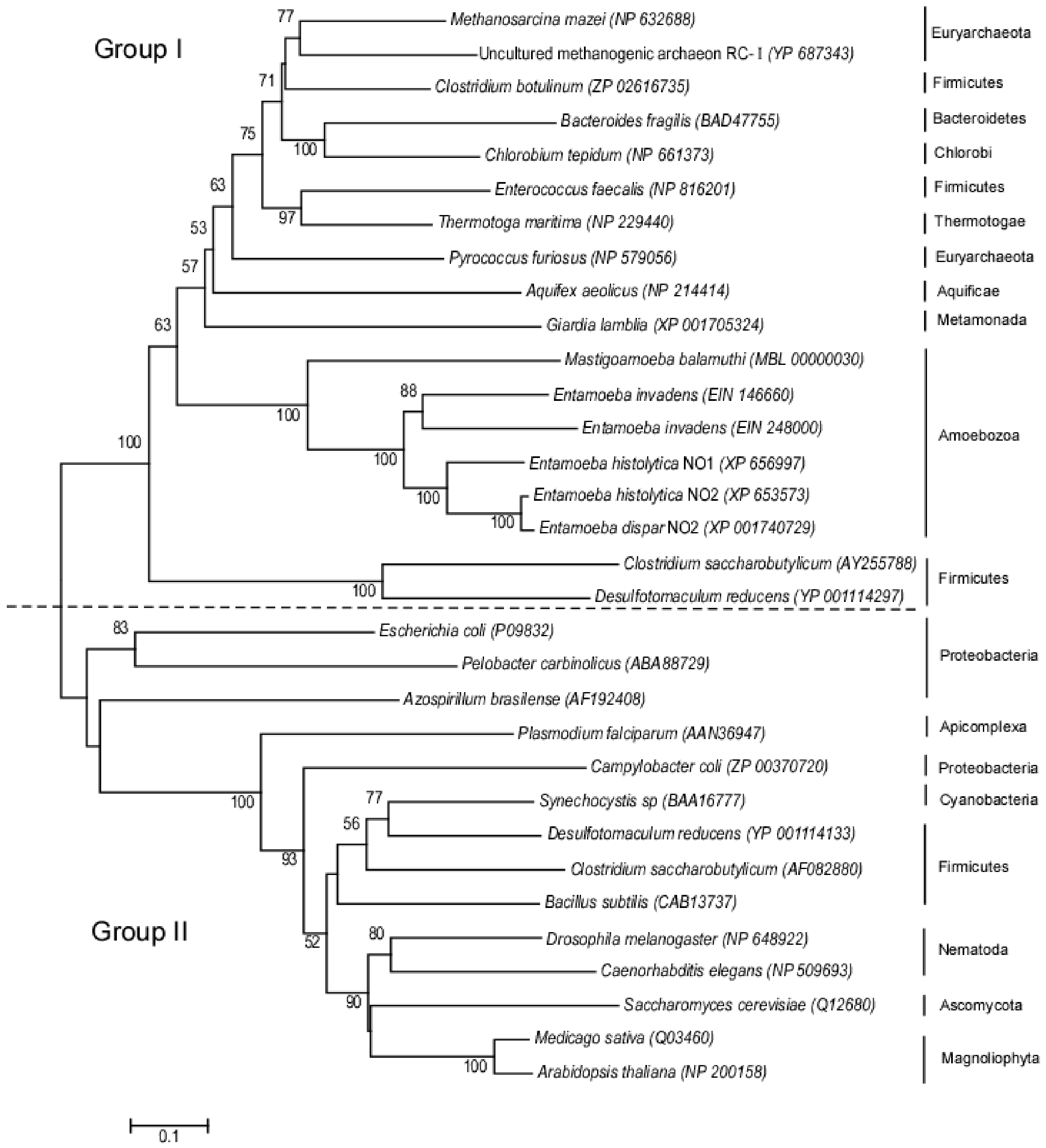
Suppl. Figure S2



Suppl. Figure S3