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Growth- and Substrate-Dependent Transcription of the Formate Dehydrogenase (*fdhCAB*) Operon in *Methanobacterium thermoformicicum* Z-245

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The formate dehydrogenase-encoding *fdhCAB* operon and flanking genes have been cloned and sequenced from *Methanobacterium thermoformicicum* Z-245. *fdh* transcription was shown to be initiated 21 bp upstream from *fdhC*, although most *fdh* transcripts terminated or were processed between *fdhC* and *fdhA*. The resulting *fdhC*, *fdhAB*, and *fdhCAB* transcripts were present at all growth stages in cells growing on formate but were barely detectable during early exponential growth on H_2 plus CO_2. The levels of the *fdh* transcripts did, however, increase dramatically in cells growing on H_2 plus CO_2, coincident with the decrease in the growth rate and the onset of constant methanogenesis that occurred when culture densities reached an optical density at 600 nm of ~0.5. The *mth* transcript that encodes the H_2-dependent methenyl-H_4 MPT reductase (MTH) and the *frh* and *mvh* transcripts that encode the coenzyme F_{420}-reducing (FRH) and nonreducing (MVH) hydrogenases, respectively, were also present in cells growing on formate, consistent with the synthesis of three hydrogenases, MTH, FRH, and MVH, in the absence of exogenously supplied H_2. Reducing the H_2 supply to *M. thermoformicicum* cells growing on H_2 plus CO_2 reduced the growth rate and CH_4 production but increased *frh* and *fdh* transcription and also increased transcription of the *mtd*, *mer*, and *mcr* genes that encode enzymes that catalyze steps 4, 5, and 7, respectively, in the pathway of CO_2 reduction to CH_4. Reducing the H_2 supply to a level insufficient for growth resulted in the disappearance of all methane gene transcripts except the *mcr* transcript, which increased. Regions flanking the *fdhCAB* operon in *M. thermoformicicum* Z-245 were used as probes to clone the homologous region from the *Methanobacterium thermoautotrophicum* ΔH genome. Sequencing revealed the presence of very similar genes except that the genome of *M. thermoautotrophicum*, a methanogen incapable of growth on formate, lacked the *fdhCAB* operon.

Methanobacterium thermoautotrophicum ΔH and *Methanobacterium thermoformicicum* Z-245 are very closely related, thermophilic methanogens that could be considered members of the same species (16, 23, 41). However, *M. thermoautotrophicum* grows only by the H_2-dependent reduction of CO_2 to CH_4 (9, 39), whereas *M. thermoformicicum* is more metabolically versatile and can also catabolize formate to CH_4 and therefore can grow in the absence of exogenously supplied H_2. The availability of H_2 determines which of two intersecting pathways of CO_2 reduction to CH_4 is employed by *M. thermoautotrophicum* (20, 39). When excess H_2 is available, the *mth* and *mrt* genes are transcribed, resulting in the synthesis of the H_2-dependent N^5,N^{10}-methenyltetrahydromethanopterin (methenyl-H_4MPT) reductase (MTH) and methyl coenzyme M reductase (MR) II, respectively, that catalyze steps 4 and 7 in the seven-step pathway of CO_2 reduction to CH_4 (5, 25, 27, 49). However, under H_2-limited growth conditions, these steps are catalyzed by the reduced coenzyme F_{420}-dependent N^5,N^{10}-methenyl-H_4MPT reductase (MTD) and MRI encoded by the *mtd* and *mcr* genes, respectively (5, 21, 27). *M. thermoautotrophicum* cells synthesizing MTH and MRII grow faster than *M. thermoautotrophicum* cells synthesizing MTD and MRI, but inexplicably, cultures supplied with excess H_2 have lower growth yields (biomass synthesized per mole of methane generated [Y_{CH4}]) than cultures growing under H_2-limited conditions (20, 35). *M. thermoautotrophicum* cells synthesizing CH_4 via the MTD- and MCR-catalyzed pathway apparently couple the energy available from methanogenesis more efficiently to anabolism.

Investigations of the interrelationships of substrate availability, growth rates, growth yields, and alternative pathways from CO_2 to CH_4 in *M. thermoautotrophicum* are limited by H_2 being essential for growth under all conditions. *M. thermoformicicum* Z-245, which can also grow on formate, therefore seems like an attractive, experimentally more tractable alternative, provided that the same relationships hold in *M. thermoformicicum* Z-245. The results reported here confirm that growth rates, growth yields, methanogenesis, and methane gene transcription are similarly regulated by H_2 availability in *M. thermoformicicum* Z-245 cells growing on H_2 plus CO_2, and results are also reported that establish how these parameters respond to growth on formate.

Hydrogenase activity is required for growth on H_2 (9, 39), whereas formate dehydrogenase activity (FDH) (33, 34, 38) facilitates growth on formate. It was therefore also important to determine if the two growth substrates differentially regulate the expression of the hydrogenase- and FDH-encoding genes in *M. thermoformicicum* Z-245. Probes were available to detect hydrogenase gene transcripts from the *M. thermoautotrophicum* ΔH studies (20, 25), but probes were not immediately available for FDH-encoding transcripts in *M. thermoformicicum* Z-245. The FDH-encoding *fdhCAB* operon was therefore cloned and sequenced from *M. thermoformicicum* Z-245, and the flanking sequences were also used as probes to isolate the homologous region from the *M. thermoautotrophicum* ΔH genome. These regions of the two genomes were found to contain very similar genes, except that there was no *fdhCAB* operon in the *M. thermoautotrophicum* ΔH genome.

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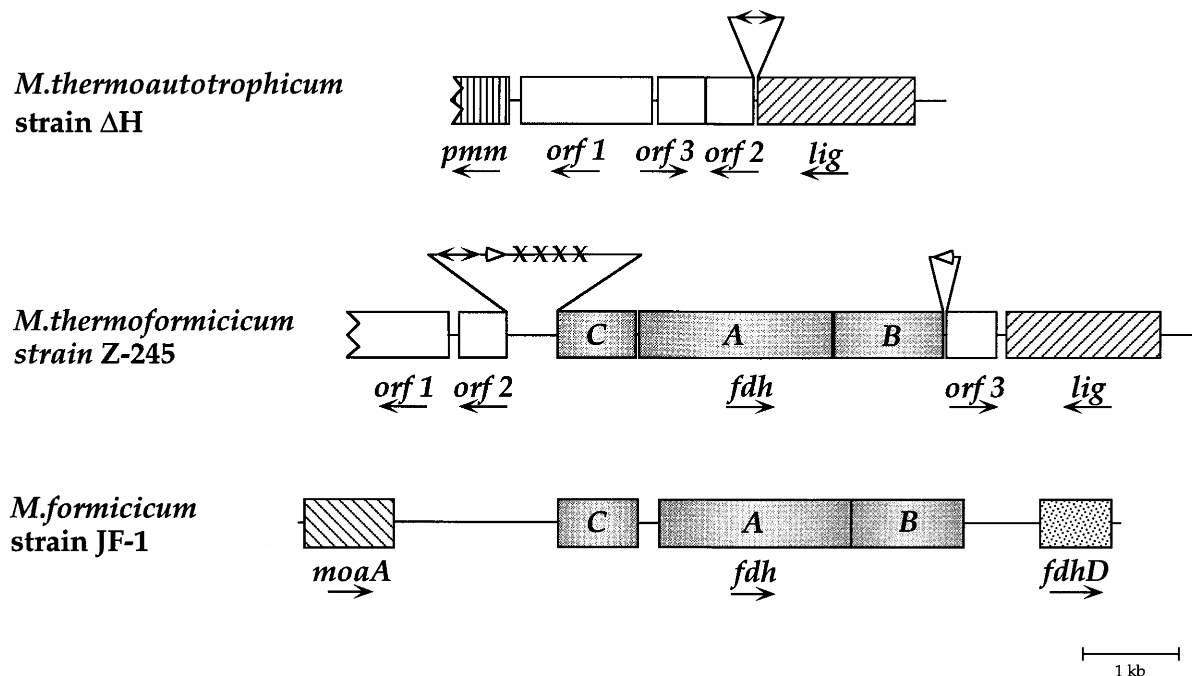

FIG. 1. Organization of the *fdhCAB* operons and flanking regions in methanogen genomic DNAs. The arrows below the genes indicate the direction of transcription. The location of an 18-bp sequence conserved 22 bp upstream of the two *orf2* genes is indicated by the diverging arrowheads, the locations of the 11-bp sequence repeated in opposite orientations flanking the *fdhCAB* operon in *M. thermoformicicum* Z-245 are indicated by the open arrowheads, and the four tandemly repeated copies of a 42-bp sequence located upstream of the *fdhCAB* operon in *M. thermoformicicum* are indicated by the X's. Shaded boxes indicate genes for which functions can be predicted based on sequence homologies. The sequences of the *M. formicicum fdhCAB* operon and flanking regions have been published (36, 44).

MATERIALS AND METHODS

Microorganisms. *M. thermoformicicum* Z-245 (DSM 3720), *M. thermoautotrophicum* ΔH (DSM 1053), *Methanobacterium wolfei* (DSM 2970), and *Methanothermus fervidus* (DSM 2088) were the strains investigated. *Escherichia coli* DH5α (12) and KW251 (Promega Corp., Madison, Wis.) were used to propagate pUC18-based recombinant plasmids (46) and as the host for λGEM11-based recombinant phages, respectively.

Growth media and conditions. Methanogens were grown in a basal salts medium (27) supplemented with 1 μM sodium tungstate. When grown on 80% H_2:20% CO_2 in serum bottles, the atmosphere was pressurized to 300 kPa, and when 100 mM sodium formate (buffered at room temperature to pH 8 by using 100 mM Tris · HCl) was supplied as the growth substrate, the atmosphere was N_2 at 50 kPa. *M. thermoformicicum* cultures were grown at 55°C, *M. thermoautotrophicum* and *M. wolfei* cultures were grown at 65°C, and *M. fervidus* cultures were grown at 83°C. For transcript analyses, 1.5-liter cultures of *M. thermoformicicum* Z-245 were grown in 2-liter fermentors (Applicon Inc., Foster City, Calif.) fitted with double reference pH electrodes connected to a pH controller unit (Cole Palmer, Chicago, Ill.). Cultures were grown on 80% H_2:20% CO_2 (200 ml/min) and maintained at pH 7.5 by the automated addition of 1 N HCl or 1 N NaOH or were grown on 100 mM sodium formate and maintained at pH 7.5 by adding 10 N formic acid (18, 33, 36). After being autoclaved, the growth media were sparged with 80% H_2:20% CO_2 or N_2 (for formate growth) for ~1 h before cysteine · HCl (0.5 g/liter) and sodium thiosulfate (0.62 g/l) were added, and the culture was inoculated. Unless stated otherwise, the impeller speed was 600 rpm for growth on H_2 plus CO_2 and 150 rpm for growth on formate.

E. coli cultures were grown at 37°C in Luria-Bertani medium (31) supplemented, when appropriate, with 50 μg of ampicillin per ml or 5 μg of tetracycline per ml.

Methane measurements. The methane content of 100-μl aliquots of the headspace gas, sampled with a gas-tight syringe, was determined by gas chromatography (7) using a Shimazu GC-8A chromatograph (Columbia, Md.) with a flame ionization detector.

Enzymes and chemicals. Enzymes used for nucleic acid manipulations were purchased from Gibco/BRL (Gaithersburg, Md.) and from Boehringer Mannheim Biochemicals (Indianapolis, Ind.). Radioactively labeled nucleotides ([α-^{32}P]dATP [110 TBq/mmol], [γ-^{32}P]ATP [259 Tbq/mmol], and [α-^{35}S]dATP [37 Tbq/mmol]) were purchased from ICN Biochemicals Inc. (Costa Mesa, Calif.). All other reagents were purchased from Sigma Chemical Corp. (St. Louis, Mo.) and Jenneile Enterprises (Cincinnati, Ohio).

DNA isolation, cloning, and sequencing. Methanogen genomic DNA was isolated, and a λGEM11-based library of *M. thermoformicicum* Z-245 genomic DNA was constructed, as previously described for *M. thermoautotrophicum* ΔH (25, 43). All DNA manipulations followed standard protocols (31), except that the procedure described by Meese et al. (19) was used to isolate λ DNA. Insert DNAs and linearized vector DNAs were purified by agarose gel electrophoresis and the GeneClean procedure (Bio 101, La Jolla, Calif.) prior to ligation.

The DNA from λGEM11 recombinant phages was screened by plaque hybridization (31) for *M. thermoformicicum* Z-245 sequences that hybridized to a ^{32}P-labeled ~2.2-kbp *Sph*I-*Bam*HI restriction fragment that contained the *fdhA* gene from *Methanobacterium formicicum* (36). Phage DNA was denatured and fixed to Zeta-Probe nylon membranes (Bio-Rad, Richmond, Calif.) that were incubated for 20 min at 65°C in hybridization buffer (0.25 M Na_2PO_4, 7% [wt/vol] sodium dodecyl sulfate [SDS], 1 mM EDTA, 1% [wt/vol] bovine serum albumin) before denatured, ^{32}P-labeled probe was added. After hybridizations for ~16 h at 50°C, the membranes were washed twice for 30 min in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M Na citrate) that contained 0.1% SDS, either at 50°C (medium stringency) or at 65°C (stringent conditions). DNA was isolated from three positive clones, designated λZ1, λZ2, and λZ3, and the restriction fragments that contained the *M. thermoformicicum* Z-245 *fdh* genes were identified by Southern blot hybridizations (31). The ^{32}P-labeled *fdhA*-containing restriction fragment and a ^{32}P-labeled ~1.6-kbp *fdhB*-containing *Bam*HI fragment of *M. formicicum* DNA were used as probes (36). Restriction fragments that gave positive signals were subcloned into pUC18 and sequenced by using the dideoxy chain terminating technology (32) with the 7-deaza version of the Sequenase 2.0 kit (USB, Cleveland, Ohio). Double-stranded DNA templates (47) were sequenced by using oligonucleotide primers complementary to either the vector or adjacent insert sequences. The University of Wisconsin GCG software version 8.1 and the BLAST program (2) were used for sequence analyses.

Plasmid pZ3-1 was constructed by ligating a 5.5-kbp *Sst*I-*Xho*I fragment from λZ3 into pUC18, which contained the 3′ terminus of the *M. thermoformicicum fdhA* gene, the *fdhB* gene, and downstream sequences (Fig. 1). This was used as the probe to screen a λGEM11-based library of *M. thermoautotrophicum* ΔH genomic DNA (25) for homologous sequences. The DNAs from three phages that gave positive hybridization signals, designated λH11, λH12, and λH13, were then screened by Southern blot hybridizations for sequences homologous to sequences upstream of the *M. thermoformicicum fdhC* gene (44). Plasmid pZ1-4, constructed by cloning a 2.5-kbp *Xho*I-*Hin*dIII fragment from λZ1 that contained the 5′ terminus of the *M. thermoformicicum fdhC* gene and upstream sequences, was used as the probe. An ~5-kbp *Sal*I fragment from λH12 that hybridized to both pZ3-1 and pZ1-4 was cloned into pUC18, generating plasmid pH12-1, and sequenced.

RNA isolation, Northern blotting, and primer extensions. RNA preparations, isolated from aliquots of fermentor-grown *M. thermoformicicum* cells, were analyzed by Northern blot and primer extension procedures as previously described

(20, 27). The same probes were used to identify the *M. thermoformicicum* Z-245 *frh* (coenzyme F_{420}-reducing hydrogenase), *ftr* (formylmethanofuran:H_4MPT formyltransferase), *ftsA* (coenzyme F_{390} synthetase), *mer* (coenzyme F_{420}-dependent methylene-H_4MPT reductase), *mcr* (methyl-coenzyme M reductase I), *mth* (H_2-dependent methenyl-H_4MPT reductase), *mtd* (reduced coenzyme F_{420}-dependent methenyl-H_4MPT reductase), *mtr* (methyl-H_4MPT:coenzyme M methyltransferase), *mrt* (methyl-coenzyme M reductase II), and *mvh* (methyl viologen-reducing hydrogenase) transcripts that were used to detect the homologous *M. thermoautotrophicum* ΔH transcripts (1, 20, 21, 24, 27, 28). Oligonucleotides with the sequences 5′GATGTCTCTTCCAGGGTTCCACACC, 5′AACAGGTTTTGCCGTATCTGCAGG, and 5′TGTGTCCATGCAATGTTACGATTGCG, complementary to transcripts of the nucleotides between positions 88 to 113, 21 to 46, and 87 to 112 of the *fdhA*, *fdhC*, and *orf3* genes were used as probes to detect transcripts of the *fdhCAB* operon and of *orf3* (Fig. 1), and also as primers in primer extension reactions. Probes for Northern blot hybridizations were ^{32}P end labeled with [γ-^{32}P]dATP or were labeled during PCR amplification with both ^{32}P-end-labeled primers and by the incorporation of [α-^{32}P]dATP (20, 31). The hybridization conditions and procedures used for washing, probe removal, and reprobing have been described (20).

Nucleotide sequence accession numbers. The sequence of the *fdhCAB* operon and flanking regions determined from *M. thermoformicicum* Z-245 has GenBank accession number U52681, and the sequence of the homologous region from the *M. thermoautotrophicum* ΔH genome has GenBank accession number U51624.

RESULTS

Structure of the *M. thermoformicicum* Z-245 *fdhCAB* region and comparison with the homologous region of the *M. thermoautotrophicum* ΔH genome. The genes and open reading frames (ORFs) in 9,110 and 5,066 bp of DNA sequenced from the *M. thermoformicicum* Z-245 and *M. thermoautotrophicum* ΔH genomes, respectively, are illustrated and compared in Fig. 1 with the genes in the *fdh* region of the *M. formicicum* JF-1 genome (26, 36, 44). The *M. thermoformicicum* Z-245 DNA contained an ~4.7-kbp central region, formed by ~600 bp of noncoding sequence, upstream from three closely linked ORFs designated the *fdhCAB* operon that was absent from the *M. thermoautotrophicum* ΔH DNA. The *M. thermoformicicum* Z-245 *fdhCAB* genes are predicted to encode polypeptides that contain 274, 681, and 394 amino acid residues (calculated M_rs of 29.1, 75.2, and 43.7 kDa, respectively) with sequences that are 80, 67, and 70% identical, respectively, to the sequences of the FdhCAB polypeptides encoded by the *fdhCAB* operon in *M. formicicum*. FdhA and FdhB are the subunits of FDH, and FdhC is thought to participate in formate uptake in *M. formicicum* (3, 18, 36, 44). In the *M. thermoformicicum fdhCAB* operon, 37 bp separate the *fdhC* and *fdhA* genes, and 11 bp separate the *fdhA* and *fdhB* genes, whereas the intergenic region between *fdhC* and *fdhA* is 235 bp long and the *fdhA* and *fdhB* genes overlap by 2 bp in *M. formicicum* (Fig. 1) (36, 44). Sequences consistent with strong ribosome binding sites (6) precede the three *M. thermoformicicum fdh* genes, and 258 bp upstream of *fdhC*, within the noncoding region that is not present in the *M. thermoautotrophicum* ΔH genome, are four tandemly repeated copies of a 42-bp sequence. There is no obvious conservation of sequences in the regions upstream of the *fdhC* genes in *M. thermoformicicum* and *M. formicicum*, except for the palindromic sequence 5′TTAATTAA that is appropriately positioned in both upstream regions to be the TATA-box element (13) of the *fdh* promoter (26, 44; see below).

Homologs of the ORFs upstream and downstream from the *M. thermoformicicum fdhCAB* operon are present in the *M. thermoautotrophicum* ΔH DNA, although in a different arrangement (Fig. 1). The most 5′ ORF, designated *orf1*, truncated in the DNA cloned from *M. thermoformicicum*, encodes a sequence of 360 amino acids that is 95% identical to the sequence of amino acids encoded by the homologous region of the *orf1* from *M. thermoautotrophicum* ΔH. The complete *orf1* sequence obtained from *M. thermoautotrophicum* predicts a gene product that is a basic polypeptide (pI of ~10) with 465 amino acid residues in a sequence unrelated to sequences in the databases. Upstream of *orf1* in *M. thermoautotrophicum* is the 3′ terminus of an ORF designated the *pmm* gene, as it encodes an amino acid sequence 35% identical to the sequence of phosphomannomutase encoded by the *pmm* gene in *E. coli* (15). Downstream of *orf1* in *M. thermoautotrophicum* are two oppositely oriented ORFs, with overlapping translation terminating codons, organized *orf3-orf2*. In *M. thermoformicicum*, *orf3* and *orf2* are separated by the 4.7-kbp *fdhCAB*-containing region and their order is reversed, resulting in an *orf2*-(*fdhCAB*)-*orf3* arrangement; however, their opposite orientation is retained. Located 22 bp upstream of *orf2* in both methanogens is a conserved 18-bp sequence which contains the palindrome 5′TATTTAAATA. If the *fdhCAB*-containing region was deleted from the *M. thermoautotrophicum* genome or inserted into the *M. thermoformicicum* genome, this appears to have occurred between this 18-bp sequence and the 42-bp repeats that are present only in the *M. thermoformicicum* genome. Adumbrating sites for this event, the sequence 5′AAAAAtAGAAT is present between the 18-bp and 42-bp sequences and, except for a G at the position indicated by the t, is repeated in the opposite orientation 11 bp downstream from *fdhB*, in the intergenic region that separates *fdhB* and *orf3* (Fig. 1).

The *orf2* nucleotide sequences are 66% identical and encode amino acid sequences that are 64% identical but not obviously related to sequences in the databases. The two *orf3* nucleotide sequences are 62% identical and are predicted to encode ~19-kDa polypeptides with sequences that are 52% identical and which contain a central region of ~50 amino acids with sequences ~53% identical to sequences characteristically conserved in prokaryotic group A carbonic anhydrases (11).

At 59 bp 3′ from *orf3* in *M. thermoformicicum* and 35 bp 5′ from *orf2* in *M. thermoautotrophicum* are termination codons of ORFs that encode 557 and 561 amino acids, respectively, with sequences that are 95% identical. These sequences are also ~36% and ~33% identical to the amino acid sequences of DNA ligases from *Desulfurolobus ambivalens* and *Saccharomyces cerevisiae*, respectively (17), and contain all of the motifs conserved in ATP-dependent DNA ligases. These ORFs have therefore been designated *lig* genes, as they almost certainly encode the DNA ligases of the two methanogens.

Growth and methanogenesis of *M. thermoformicicum* Z-245 on H_2 plus CO_2 and on formate. *M. thermoformicicum* Z-245 cells growing in fed-batch cultures on H_2 plus CO_2 had generation times of ~2.1 h, and cultures reached a final optical density at 600 nm (OD_{600}) of ~3, whereas cells growing in the same fermentors on formate with a N_2 atmosphere had generation times of ~4 h and cultures reached a maximum OD_{600} of ~1 (Fig. 2). Methane production by the H_2-plus-CO_2-grown cultures increased exponentially up to an OD_{600} of ~0.5 and then became constant, as documented for *M. thermoautotrophicum* cultures grown on H_2 plus CO_2 (20, 35). Growth, however, continued at an only slightly reduced rate, and these cultures therefore then grew with a continuously improving growth yield (biomass synthesized per mole of methane generated [Y_{CH4}]). Methane production by formate-grown cultures also increased exponentially, in parallel with the increase in biomass, until the culture density reached an OD_{600} of ~0.5. Methanogenesis then decreased ~40% and became constant, and the biomass in these cultures increased only very slowly after methanogenesis became constant (Fig. 2).

Transcription of the *fdhCAB* operon in *M. thermoformicicum* Z-245 cells growing on H_2 plus CO_2 and on formate. Transcripts of the *fdhCAB* operon were identified on Northern blots of RNA preparations isolated from *M. thermoformicicum* cells

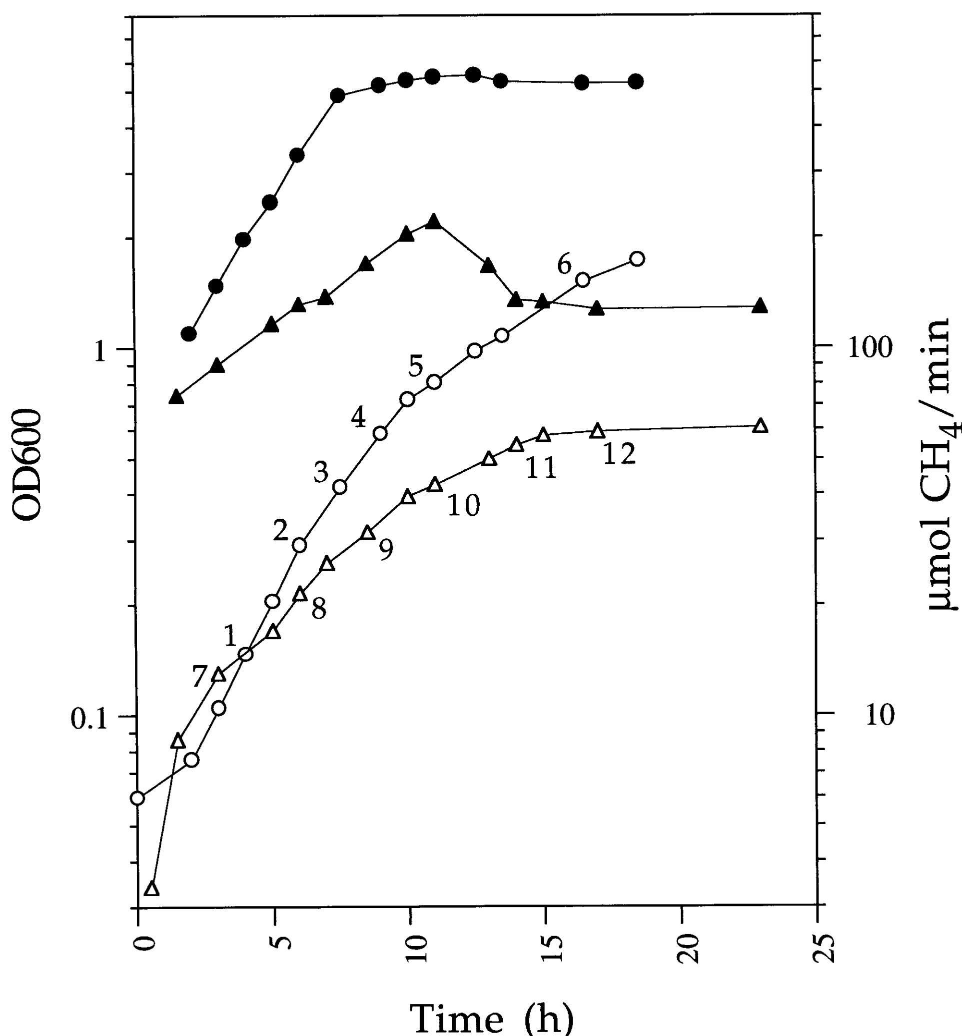

FIG. 2. Growth and methane generation by *M. thermoformicicum* Z-245 cultures. The growth (open symbols) and methane production (closed symbols) by *M. thermoformicicum* cultures growing on H_2 plus CO_2 (circles) or on formate (triangles) are shown. RNA preparations were isolated from these cultures sampled at the time points indicated 1 to 6 (growth on H_2 plus CO_2) and 7 to 12 (growth on formate).

grown on formate and grown on H_2 plus CO_2. The *fdhC*-specific probe hybridized strongly to an ~0.9-kb transcript and less intensely to an ~4.2-kb transcript (Fig. 3). The *fdhA*-specific probe also hybridized to the ~4.2-kb transcript and to an ~3.3-kb transcript but did not hybridize to the ~0.9-kb transcript (Fig. 3). A simple interpretation of these results is that most *fdh* transcripts, initiated upstream of *fdhC*, either terminated or were processed between *fdhC* and *fdhA* (see diagram in Fig. 3). Consistent with this, the sequence 5′TTA ATTTATTTTTT located within the *fdhCA* intergenic region resembles sequences known to signal transcription termination in methanogens (6, 40). However, a weak promoter in the *fdhCA* intergenic region could also direct the initiation of the ~3.3-kb *fdhAB* transcript. Hybridization signals were not obtained with the *orf3*-specific probe, indicating that the *fdhCAB* transcripts did not extend into *orf3*, which is located 32 bp downstream from *fdhB* in the *M. thermoformicicum* genome (Fig. 1).

All three *fdh* transcripts were readily detectable in all RNA preparations isolated from formate-grown cells, whereas the *fdh* transcripts were barely detectable in RNA preparations isolated during the early exponential growth phase from *M. thermoformicicum* cells growing on H_2 plus CO_2 (Fig. 3). The *fdh* transcripts, however, increased dramatically in abundance coincident with the decrease in growth rate and the onset of constant methanogenesis that occurred when cultures grown on H_2 plus CO_2 reached an OD_{600} of ~0.5.

Primer extension experiments, using a primer complementary to a sequence near the 5′ terminus of the *fdhC* transcript, demonstrated that transcription initiation occurred 21 bp upstream of the *fdhC* gene, both in formate-grown cells (Fig. 4) and in cells growing on H_2 plus CO_2 (22). This site is 25 bp downstream from the sequence 5′TTAATTAA, which appears therefore, as in *M. formicicum* (44), to be the TATA-box element (6, 13, 44) of the *fdhCAB* promoter in *M. thermoformicicum*. Primer extension experiments undertaken with a primer complementary to a sequence near the 5′ terminus of the *fdhA* transcript failed to generate detectable signals (22).

Growth phase-dependent transcription of methane genes in *M. thermoformicicum* Z-245. The growth phase-dependent patterns of *ftr*, *mth*, *mtd*, *mrtBDGA*, *mcrBDCGA*, *frhADGB*, *mvhDGAB*, and *ftsA* transcription, observed in *M. thermoautotro-*

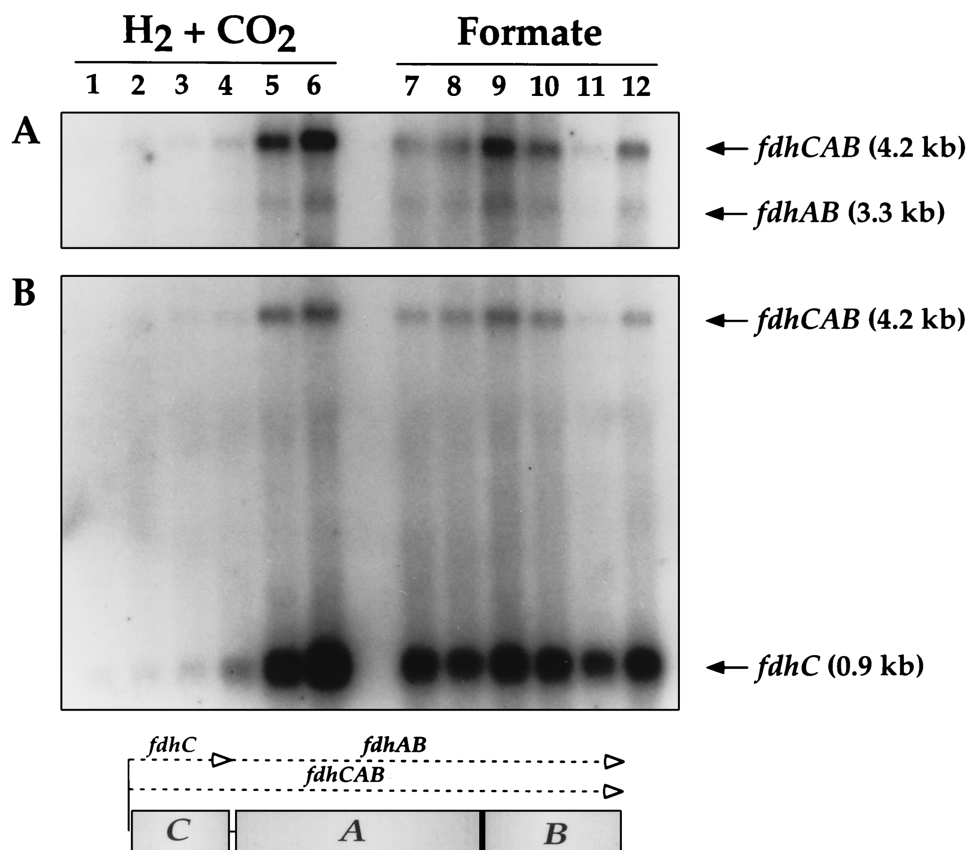

FIG. 3. Northern blot analysis of *fdh* transcripts in RNA from *M. thermoformicicum* cells grown on H_2 plus CO_2 or on formate. Probes specific for *fdhA*-containing transcripts (A) and specific for *fdhC*-containing transcripts (B) were used to probe aliquots (5 μg) of RNA preparations isolated from the cultures of *M. thermoformicicum* shown in Fig. 2, grown on H_2 plus CO_2 (sampled at time points 1 to 6) and grown on formate (sampled at time points 7 to 12). The ~0.9-kb transcript that hybridized to the *fdhC* probe, documented in panel B, did not hybridize to the *fdhA* probe (not shown). The diagram below panel B provides an interpretation of the *fdh* Northern blot data.

phicum cells grown on H_2 plus CO_2 (20, 24, 25, 27), also occurred in *M. thermoformicicum* cells growing on H_2 plus CO_2. The *mth* and *mrt* transcripts were most abundant during the early exponential growth phase and then declined when the OD_{600} reached ~0.5, coincident with the onset of constant methanogenesis and increases in the *mtd* and *mcr* transcripts (Fig. 5). The *ftsA* transcript that encodes coenzyme F_{390} synthetase (42) declined at the later growth stages but did not completely disappear, whereas *frh* transcripts that encode the coenzyme F_{420}-reducing hydrogenase (FRH) (1) increased slightly in abundance during the later growth stages. The level of the *mvh* transcript that encodes the coenzyme F_{420}-nonreactive hydrogenase (MVH) (28) remained almost constant throughout the growth of cultures on H_2 plus CO_2.

M. thermoformicicum cultures growing on formate exhibited similar but not as clearly delineated patterns of growth phase-dependent methane gene transcription (Fig. 5). The *mth* and *mrt* transcripts did decline in abundance at the end of exponential growth, but *mtd* and *mcr* transcripts were present at relatively high levels at all growth stages. As the *frh* and *mvh* transcripts were also present, all three hydrogenases, MTH, FRH, and MVH (1, 28, 39, 49), were apparently synthesized by *M. thermoformicicum* cells growing on formate. Methanogens catabolize formate to CO_2 and H_2 en route to CH_4 (3, 9, 33, 38, 45), and transcription of the *frh* and *mvh* operons would be consistent with the synthesis of FRH and MVH to metabolize the formate-derived H_2. The *mth* transcript, however, is

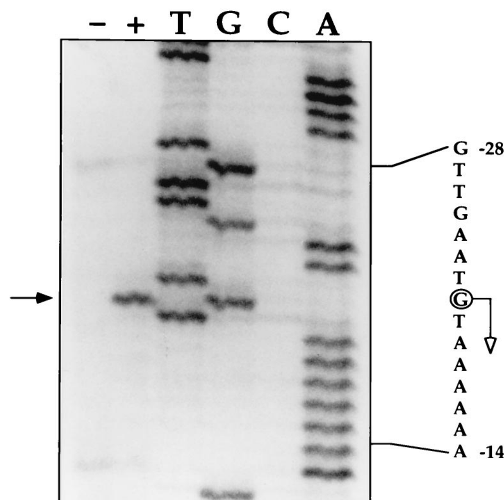

FIG. 4. Identification of the *fdh* transcription initiation site. The products of primer extension reactions incubated with (+) and without (−) reverse transcriptase are shown adjacent to the sequencing ladders that were used to determine the length of the indicated product. The primer was complementary to a sequence at the 5′ terminus of the *fdhC* gene, and the results shown were obtained with an RNA preparation isolated from *M. thermoformicicum* cells grown on formate. Experiments with RNA preparations isolated from cells grown on H_2 plus CO_2 demonstrated that *fdh* transcription initiation occurred at the same site in these cells as in formate-grown cells (22). The sequence of the region upstream of *fdh* operon is shown with the site of transcription initiation circled.

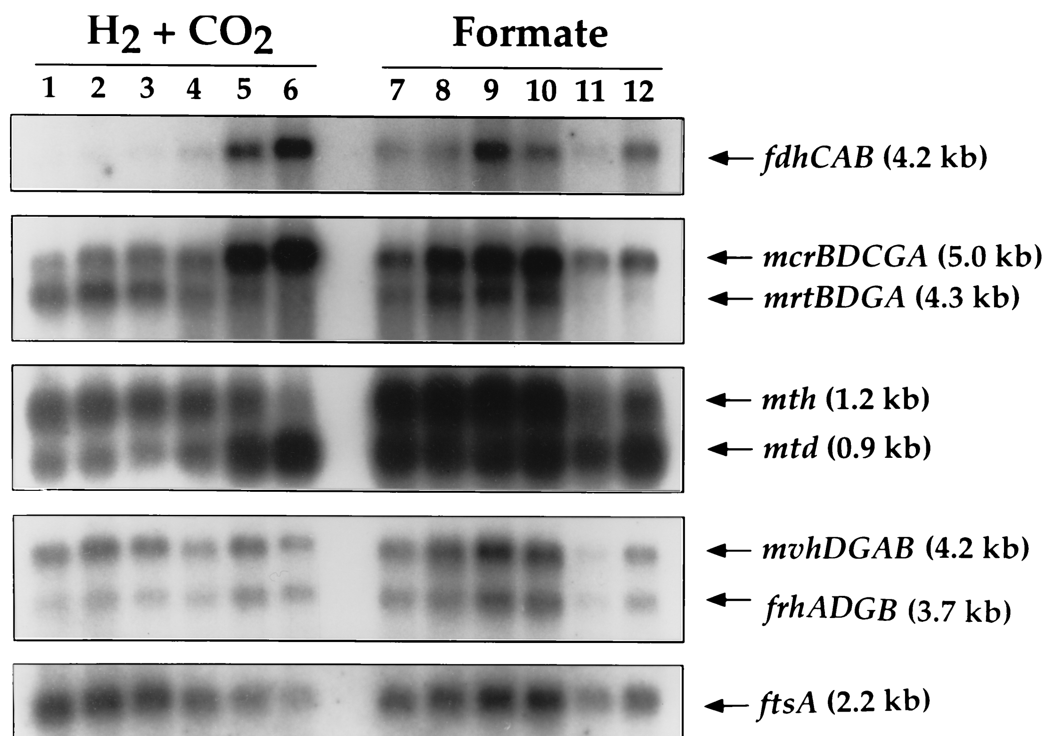

FIG. 5. Growth phase-dependent transcription of methane genes. Aliquots (5 μg) of RNA preparations isolated at time points 1 to 6 and 7 to 12, from the *M. thermoformicicum* cultures grown on H_2 plus CO_2 and grown on formate, respectively, shown in Fig. 2, were probed on Northern blots with ^{32}P-labeled probes specific for the transcripts identified to the right of the figure.

present in cells growing on H_2 plus CO_2 only under conditions of excess H_2 availability (Fig. 5) (25), and therefore the presence of *mth* transcripts in cells growing on formate was somewhat surprising (Fig. 5). The intracellular concentration of H_2 generated from formate could be sufficiently high to direct *mth* expression, although *mtd* transcripts were also present at all stages of growth on formate. The activities of both step 4-catalyzing enzymes, MTH and MTD, may therefore be needed concurrently for *M. thermoformicicum* cells to grow on formate.

The level of the *ftsA* transcript (20, 42) varied very little in RNA preparations isolated at different growth stages from *M. thermoformicicum* cells growing on formate (Fig. 5).

Regulation of methane gene transcription by impeller speed. Changing the impeller speed can be used to regulate the rate at which H_2 is supplied to cells growing in fed-batch cultures sparged with H_2 plus CO_2 (20). Reducing the impeller speed from 600 to 280 rpm reduced the growth rate of *M. thermoformicicum*, increasing the generation time to ~6.5 h, and resulted in cultures that synthesized methane at a constant and ~70% lower rate (Fig. 6B). These changes were accompanied by the disappearance of the *mth* and *mrt* transcripts, reductions in the levels of the *ftr* and *mvh* transcripts, and increases in the levels of the *mtd*, *mcr*, and *fdh* transcripts (Fig. 7).

More-severe reductions in the impeller speed, from 600 to 120 rpm, halted growth, and methanogenesis decreased to <5% of the 600-rpm rate (Fig. 6A). During incubations with the impeller speed at 120 rpm, all methane gene transcripts declined to undetectable levels except for the *mcr* transcript which, after an initial decrease, increased (Fig. 7).

Returning the impeller speed to 600 from 280 rpm restored the original rates of growth and methanogenesis (Fig. 6B) and the pattern of methane gene transcription. The *mth* and *mrt* transcripts reappeared, the *mvh* and *ftr* transcripts increased in abundance, and the levels of the *mtd*, *mcr*, and *fdh* transcripts decreased (Fig. 7). Returning the impeller speed to 600 rpm from 120 rpm similarly restored growth, methanogenesis (Fig. 6A), and the pattern of methane gene transcription present before the reduction in the impeller speed (Fig. 7). Although the *M. thermoformicicum* Z-245 cells had been incubated for >3 h with an H_2 supply insufficient for growth, they apparently remained fully viable and capable of an immediate growth response when provided with a sudden increase in the H_2 supply.

DISCUSSION

Growth of methanogens on formate is made possible by the presence of FDH (3, 9, 18, 34, 38), and the FDH-encoding *fdhCAB* operon from *M. formicicum* has been studied in detail (26, 36, 44). Here we have determined the primary sequence and regulation of expression of a related *fdhCAB* operon from *M. thermoformicicum* Z-245 (Fig. 1). Their conserved organization and similar primary sequences indicate that these two *fdhCAB* operons share a common ancestry (23), and Southern blot hybridizations have revealed that *fdhCAB*-related sequences are also present in the genomes of *M. wolfei* and *M. fervidus* (22). *M. wolfei* has, in fact, also been shown to grow and to exhibit its characteristic autolysis on formate under an atmosphere of N_2, although several attempts to grow *M. fervidus* on formate were unsuccessful (22). Southern blot hybridizations, in contrast, indicated that *M. thermoautotrophicum* ΔH and Marburg did not contain closely related *fdhCAB* sequences, and this negative result was substantiated by determining the sequence of the *fdh* region from the *M. thermoautotrophicum* ΔH genome. Although the genes that flank the

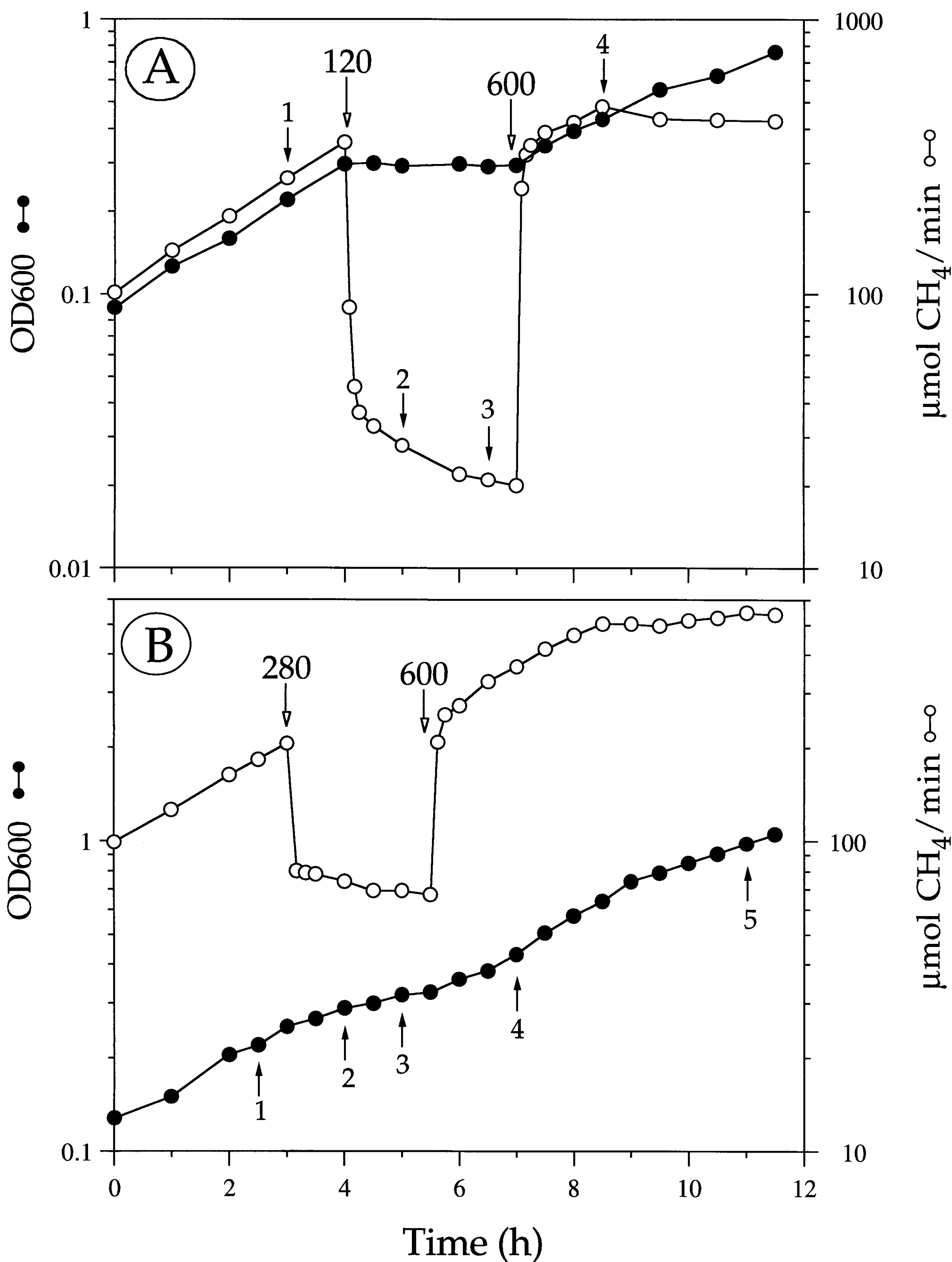

FIG. 6. Impeller speed regulation of growth and methanogenesis. The growth (●) and methane production (○) of two *M. thermoformicicum* cultures growing initially with an impeller speed of 600 rpm are shown. At the points indicated by the open-headed arrows, the impeller speed was reduced to 120 rpm (A) or to 280 rpm (B) and then returned to 600 rpm. RNA preparations were isolated from these cultures at time points 1 to 4 (A) and 1 to 5 (B).

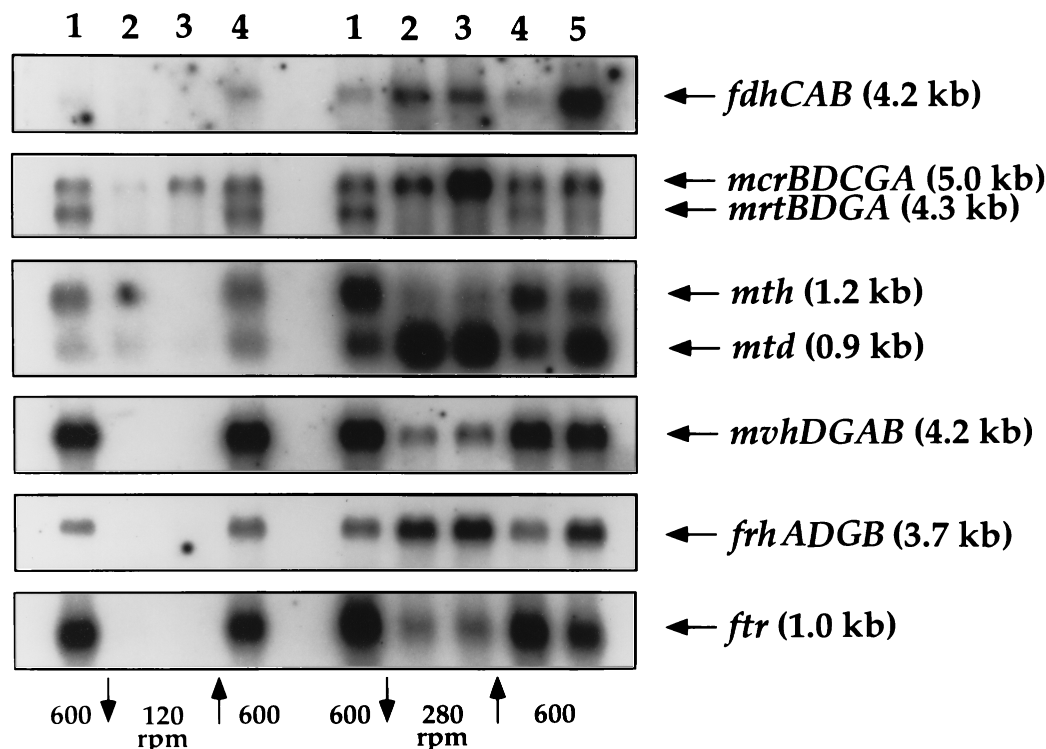

FIG. 7. Impeller speed regulation of methane gene transcription. Aliquots (5 μg) of the RNA preparations isolated from the cultures shown in Fig. 6, at time points 1 to 4 (Fig. 6A) and 1 to 5 (Fig. 6B), were probed on the Northern blots shown with ^{32}P-labeled probes specific for the transcripts identified to the right of the figure. The impeller speeds and shifts in impeller speed are indicated below the autoradiograms.

fdhCAB operon in the *M. thermoformicicum* Z-245 genome are present at this location in the *M. thermoautotrophicum* ΔH genome, there is no *fdhCAB* operon (Fig. 1), and the *M. thermoautotrophicum* ΔH genome sequencing project has confirmed that *fdhCAB*-related sequences are not present elsewhere in this genome (37). As many members of the *Methanobacteriales* can catabolize formate (4, 10, 16, 23, 33, 41, 45), *fdhCAB* operons must be widely distributed within this order, and the most parsimonious argument would seem to be that the *fdhCAB* operon was lost during the descent of the lineage that lead to *M. thermoautotrophicum* ΔH. Different genes, however, flank the *fdhCAB* operons in *M. formicicum* and *M. thermoformicicum* (Fig. 1), indicating that additional genomic rearrangements have occurred since the divergence of these two members of the *Methanobacteriales* and strengthening the argument that *M. thermoformicicum* strains could have evolved from a *M. thermoautotrophicum* strain that acquired a *fdhCAB* operon.

Unlike the genes that flank the *fdhCAB* operon in *M. thermoformicicum*, the genes that flank the *fdhCAB* operon in *M. formicicum* appear to encode proteins more readily associated with FDH. The *moaA* gene, located upstream from the *fdh* operon in *M. formicicum*, has sequences in common with the *moaA* gene in *E. coli* that encodes a protein involved in molybdenum metabolism (29), and *M. formicicum* FDH contains a molybdopterin cofactor (3, 18). The downstream *fdhD* gene has been so designated because it has sequences in common with the *fdhD* gene which, although its function is unknown, is closely affiliated with the *fdh* operon in *Wolinella succinogenes* (30). Although *moaA* and *fdhD* genes have not yet been identified in *M. thermoformicicum*, a *moaA* gene has been located adjacent to genes that encode a molybdopterin-dependent enzyme in *M. thermoautotrophicum* Marburg (14), and the *M. thermoautotrophicum* ΔH genome sequence contains both *moaA* and *fdhD* genes, separated as in *M. formicicum* by ~4 kbp, but not flanking genes obviously related to formate metabolism (37).

Transcription of the *fdh* operon in cells growing on H_2 plus CO_2. Growth on formate was required for FDH synthesis by a strain of *Methanococcus thermolithotrophicus* (38), whereas *M. formicicum* cells grown on H_2 plus CO_2 contain FDH (3, 33, 45). Consistent with the *M. formicicum* results, *fdh* transcripts were present in *M. thermoformicicum* cells growing on H_2 plus CO_2, but unexpectedly, their abundance increased dramatically coincident with the onset of constant methanogenesis, the disappearance of the *mth* and *mrt* transcripts, and the increases in the *mtd* and *mcr* transcripts (Fig. 3 and 5). Limiting growth by limiting the H_2 supply (Fig. 6B) also stimulated *fdh* transcription (Fig. 7), indicating that the growth phase-dependent increase in *fdhCAB* transcription (Fig. 3) probably also reflected the onset of H_2-limited growth. Reducing the H_2 supply also stimulated transcription of the *frh* operon (Fig. 7) that encodes FRH (1), consistent with the argument that increased synthesis and use of reduced coenzyme F_{420} would be an appropriate energy-conserving response for methanogens faced with a limited supply of H_2 (20, 24, 25). The increases that occur in the levels of the *mtd* and *mer* (22) transcripts when the H_2 supply is limited are also consistent with this argument, as both of these genes encode reduced coenzyme F_{420}-dependent enzymes (21, 24, 39).

Why *M. thermoformicicum* cells growing on H_2 plus CO_2 respond to a limitation in the H_2 supply with increased *fdhCAB* transcription is an intriguing question. Methanogens that can catabolize formate have been shown to synthesize formate when grown on H_2 plus CO_2 (10, 45), but this has not been

recognized specifically as a response to an H_2 limitation. FDH is also a coenzyme F_{420}-dependent enzyme (3, 18, 34), and the increase in *fdh* transcription could be part of a comprehensive response that increases the expression of all methanogenesis-related genes that encode coenzyme F_{420}-dependent enzymes when the H_2 supply becomes limited. Alternatively, increasing *fdhCAB* transcription could be a specific survival response, designed to increase the availability of enzymes that would allow growth to continue if formate were available when the H_2 supply was exhausted. The synthesis of formate from H_2 and CO_2 (10, 45), when conditions are inappropriate for growth, could also allow the methanogen to capture any available H_2 in a chemical form that could be stored for future consumption, as formate dissolved in the cells' immediate environment (48).

M. formicicum cells growing on formate can synthesize H_2 (33, 45), and this is likely also to be the case for *M. thermoformicicum*, as the hydrogenase-encoding *mth*, *frh*, and *mvh* genes (1, 25, 28) are transcribed in *M. thermoformicicum* cells growing on formate (Fig. 5). If increased *fdhCAB* transcription is a survival response of cells growing on H_2 plus CO_2 faced with an H_2 limitation, then transcription of the hydrogenase-encoding genes might increase in formate-growing cells faced with a formate limitation. This can be investigated directly by using formate to limit growth in a continuous culture system (8). The *fdh* transcripts are, however, present only at low levels in *M. thermoformicicum* cells growing on H_2 plus CO_2 under conditions of excess H_2, whereas the *frh*, *mvh*, and *mth* transcripts are present at relatively high levels in *M. thermoformicicum* cells growing with excess formate (Fig. 5). It seems more likely, therefore, that the three hydrogenases, FRH, MVH, and MTH, are required by *M. thermoformicicum* for growth on formate, even in the absence of exogenously supplied H_2.

Use of the impeller shock procedure. Reducing the impeller speed from 600 to 120 rpm halted the growth of *M. thermoformicicum* cells and led to the disappearance of all methane gene transcripts except for the *mcr* transcript, which increased (Fig. 7). The *mcr* promoter, therefore, appears to be unique in being activated, as in *M. thermoautotrophicum* ΔH (20), by a growth-inhibiting reduction in the H_2 supply. Archaeal transcription factors (40) are likely to activate this stress response, and their identification and isolation should be facilitated by the impeller shock procedure. Less-severe reductions in the H_2 supply, resulting from shifts in the impeller speed from 600 to 280 rpm, increased *mtd*, *mer*, *frh*, and *fdh* transcription (Fig. 7) (22). The transcription factors that activate these promoters and the intracellular signal, possibly the level of coenzyme F_{390} (24, 25, 42), that communicates a reduction in the availability of H_2 to the gene regulatory system should also now be experimentally accessible.

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REFERENCES

1. **Alex, L. A., J. N. Reeve, W. H. Orme-Johnson, and C. T. Walsh.** 1990. Cloning, sequence determination, and expression of the genes encoding the subunits of the nickel-containing 8-hydroxy-5-deazaflavin reducing hydrogenase from *Methanobacterium thermoautotrophicum* ΔH. Biochemistry **29:** 7237–7244.
2. **Altschul, S. F., W. Gish, W. Miller, E. F. Myers, and D. J. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
3. **Baron, S. F., and J. G. Ferry.** 1989. Reconstitution and properties of a coenzyme F_{420}-mediated formate hydrogenlyase system in *Methanobacterium formicicum*. J. Bacteriol. **171:**3854–3859.
4. **Benstead, J., D. B. Archer, and D. Lloyd.** 1991. Formate utilization by members of the genus *Methanobacterium*. Arch. Microbiol. **156:**34–37.
5. **Bonacker, L. G., S. Baudner, and R. K. Thauer.** 1992. Differential expression of the two methyl-coenzyme M reductases in *Methanobacterium thermoautotrophicum* as determined immunochemically via isoenzyme-specific antisera. Eur. J. Biochem. **206:**87–92.
6. **Brown, J. W., C. J. Daniels, and J. N. Reeve.** 1989. Gene structure, organization and expression in archaebacteria. Crit. Rev. Microbiol. **16:**287–338.
7. **Cao, X., and J. A. Kryzcki.** 1991. Acetate-dependent methylation of two corrinoid proteins in extracts of *Methanosarcina barkeri*. J. Bacteriol. **173:** 5439–5448.
8. **Chua, H. B., and J. P. Robinson.** 1983. Formate-limited growth of *Methanobacterium formicicum* in steady-state cultures. Arch. Microbiol. **135:**158–160.
9. **Deppenmeier, U., V. Müller, and G. Gottschalk.** 1996. Pathways of energy conservation in methanogenic bacteria. Arch. Microbiol. **165:**149–163.
10. **Eguchi, S. Y., N. Nishio, and S. Nagai.** 1985. Formic acid production from H_2 and bicarbonate by a formate-utilizing methanogen. Appl. Microbiol. Biotechnol. **22:**148–151.
11. **Fukuzawa, H., E. Suzuki, Y. Komukai, and S. Miyachi.** 1992. A gene homologous to chloroplast carbonic anhydrase (*icfA*) is essential to photosynthetic carbon dioxide fixation by *Synechococcus* PCC7942. Proc. Natl. Acad. Sci. USA **89:**4437–4441.
12. **Hanahan, D.** 1983. Techniques for transformation of *E. coli*, p. 109–136. *In* D. M. Glover (ed.), DNA cloning, vol. 1. IRL Press, Oxford, United Kingdom.
13. **Hausner, W., G. Frei, and M. Thomm.** 1991. Control regions of an archaeal gene: a TATA box and an initiator element promote cell-free transcription of the $tRNA^{val}$ gene of *Methanococcus vannielii*. J. Mol. Biol. **222:**495–508.
14. **Hochheimer, A., R. A. Schmitz, R. K. Thauer, and R. Hedderich.** 1995. The tungsten formylmethanofuran dehydrogenase from *Methanobacterium thermoautotrophicum* contains sequence motifs characteristic for enzymes containing molybdopterin dinucleotide. Eur. J. Biochem. **234:**910–920.
15. **Jayaratne, P., D. Bronner, P. R. MacLachlan, C. Dodgson, N. Kido, and C. Whitfield.** 1994. Cloning and analysis of duplicated *rfbM* and *rfbK* genes involved in the formation of GDP-mannose in *Escherichia coli* O9:K30 and participation of *rfb* genes in the synthesis of the group I K30 capsular polysaccharide. J. Bacteriol. **176:**3126–3139.
16. **Jones, W. J., D. P. Nagle, Jr., and W. B. Whitman.** 1987. Methanogens and the diversity of archaebacteria. Microbiol. Rev. **51:**135–177.
17. **Kletzin, A.** 1992. Molecular characterization of a DNA ligase gene of the extremely thermophilic archaeon *Desulfurolobus ambivalens* shows phylogenetic relationships to eukaryotic ligases. Nucleic Acids Res. **20:**5389–5396.
18. **May, H. D., P. S. Patel, and J. G. Ferry.** 1988. Effects of molybdenum and tungsten on synthesis and composition of formate dehydrogenase in *Methanobacterium formicicum*. J. Bacteriol. **170:**3384–3389.
19. **Meese, E., S. Olson, L. Leis, and J. Trent.** 1990. Quick method for the high yield of λ bacteriophage DNA. Nucleic Acids Res. **18:**1923.
20. **Morgan, R. M., T. D. Pihl, J. Nölling, and J. N. Reeve.** 1997. Hydrogen regulation of growth, growth yields, and methane gene transcription in *Methanobacterium thermoautotrophicum* ΔH. J. Bacteriol. **179:**889–898.
21. **Mukhopadhyay, B., E. Purwantini, T. D. Pihl, J. N. Reeve, and L. Daniels.** 1995. Cloning, sequencing, and transcriptional analysis of the coenzyme F_{420}-dependent methylene-5,6,7,8-tetrahydromethanopterin dehydrogenase gene from *Methanobacterium thermoautotrophicum* strain Marburg and functional expression in *Escherichia coli*. J. Biol. Chem. **270:**2827–2832.
22. **Nölling, J.** 1996. Unpublished data.
23. **Nölling, J., D. Hahn, W. Ludwig, and W. M. deVos.** 1993. Phylogenetic analysis of thermophilic *Methanobacterium* sp.: evidence for a formate-utilizing ancestor. Syst. Appl. Microbiol. **16:**208–215.
24. **Nölling, J., T. D. Pihl, and J. N. Reeve.** 1995. Cloning, sequencing, and growth phase-dependent transcription of the coenzyme F_{420}-dependent N^5,N^{10}-methylenehydromethanopterin reductase-encoding genes from *Methanobacterium thermoautotrophicum* ΔH and *Methanopyrus kandleri*. J. Bacteriol. **177:**7238–7244.
25. **Nölling, J., T. D. Pihl, A. Vriesema, and J. N. Reeve.** 1995. Organization and growth phase-dependent transcription of methane genes in two regions of the *Methanobacterium thermoautotrophicum* genome. J. Bacteriol. **177:**2460–2468.
26. **Patel, P. S., and J. G. Ferry.** 1988. Characterization of the upstream region of the formate dehydrogenase operon of *Methanobacterium formicicum*. J. Bacteriol. **170:**3390–3395.
27. **Pihl, T. D., S. Sharma, and J. N. Reeve.** 1994. Growth phase-dependent transcription of the genes that encode the two methyl coenzyme M reductase isoenzymes and N^5-methyltetrahydromethanopterin:coenzyme M methyl transferase in *Methanobacterium thermoautotrophicum* ΔH. J. Bacteriol. **176:** 6384–6391.
28. **Reeve, J. N., G. S. Beckler, D. S. Cram, P. T. Hamilton, J. W. Brown, J. A. Kryzcki, A. F. Kolodziej, L. Alex, W. H. Orme-Johnson, and C. T. Walsh.** 1989. A hydrogenase-linked gene in *Methanobacterium thermoautotrophicum* strain ΔH encodes a polyferredoxin. Proc. Natl. Acad. Sci. USA **86:**3031–3035.

29. **Rivers, S. L., E. McNairn, F. Blasco, G. Giordano, and D. H. Boxer.** 1993. Molecular genetic analysis of the *moa* operon of *Escherichia coli* K-12 required for molybdenum cofactor biosynthesis. Mol. Microbiol. **8:**1071–1081.
30. **Robinson, K., W. Gilbert, and G. M. Church.** 1994. Large scale bacterial gene discovery by similarity search. Nature Genet. **7:**205–214.
31. **Sambrook, J. E., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
32. **Sanger, F., S. Nicklen, and A. R. Coulsen.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
33. **Schauer, N. L., and J. G. Ferry.** 1980. Metabolism of formate in *Methanobacterium formicicum*. J. Bacteriol. **142:**800–807.
34. **Schauer, N. L., and J. G. Ferry.** 1982. Properties of formate dehydrogenase in *Methanobacterium formicicum*. J. Bacteriol. **150:**1–7.
35. **Schönheit, P., J. Moll, and R. K. Thauer.** 1980. Growth parameters (K_s, μ_{max}, Y_s) of *Methanobacterium thermoautotrophicum*. Arch. Microbiol. **127:** 59–65.
36. **Schuber, A. P., E. C. Orr, M. A. Recny, P. F. Schendel, H. D. May, N. L. Schauer, and J. G. Ferry.** 1986. Cloning, expression, and nucleotide sequence of the formate dehydrogenase genes from *Methanobacterium formicicum*. J. Biol. Chem. **261:**12942–12947.
37. **Smith, D., J. Nölling, and J. N. Reeve.** 1996. Unpublished data.
38. **Sparling, R., and L. Daniels.** 1990. Regulation of formate dehydrogenase activity in *Methanococcus thermolithotrophicus*. J. Bacteriol. **172:**1464–1469.
39. **Thauer, R. K., R. Hedderich, and R. Fischer.** 1993. Reactions and enzymes involved in methanogenesis from CO_2 and H_2, p. 209–252. *In* J. M. Ferry (ed.), Methanogenesis, ecology, physiology, biochemistry and genetics. Chapman and Hall, New York, N.Y.
40. **Thomm, M., W. Hausner, and C. Hethke.** 1994. Transcription factors and termination of transcription in *Methanococcus*. Syst. Appl. Microbiol. **16:** 648–655.
41. **Touzel, J. P., E. Conway de Macario, J. Nölling, W. M. de Vos, T. Zhilina, and A. M. Lysenko.** 1990. DNA relatedness among some thermophilic members of the genus *Methanobacterium*: emendation of the species *Methanobacterium thermoautotrophicum* and rejection of *Methanobacterium thermoformicicum* as a synonym of *Methanobacterium thermoautotrophicum*. Int. J. Syst. Bacteriol. **42:**408–411.
42. **Vermeij, P., R. J. T. van der Steen, G. D. Vogels, and T. Leisinger.** 1996. Coenzyme F_{390} synthetase from *Methanobacterium thermoautotrophicum* Marburg belongs to the superfamily of adenylate-forming enzymes. J. Bacteriol. **178:**505–510.
43. **Weil, C. F., D. S. Cram, B. A. Sherf, and J. N. Reeve.** 1988. Structure and comparative analysis of the genes encoding component C of the methyl coenzyme M reductase in the extremely thermophilic archaebacterium *Methanothermus fervidus*. J. Bacteriol. **170:**4718–4726.
44. **White, W. B., and J. G. Ferry.** 1992. Identification of formate dehydrogenase-specific mRNA species and nucleotide sequence of the *fdhC* gene in *Methanobacterium formicicum*. J. Bacteriol. **174:**4997–5004.
45. **Wu, W.-M., R. F. Hickey, M. K. Jahn, and J. G. Zeikus.** 1993. Energetics and regulation of formate and hydrogen metabolism by *Methanobacterium formicicum*. Arch. Microbiol. **159:**57–65.
46. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and the host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33:**103–119.
47. **Yie, Y., Z. Wei, and P. Tien.** 1993. A simplified and reliable protocol for plasmid DNA sequencing: fast miniprep and denaturation. Nucleic Acids Res. **21:**361.
48. **Zinder, S. H.** 1993. Physiological ecology of methanogens, p. 128–206. *In* J. M. Ferry (ed.), Methanogenesis, ecology, physiology, biochemistry and genetics. Chapman and Hall, New York, N.Y.
49. **Zirngibl, C., W. van Dongen, B. Schwörer, R. van Bünan, M. Richter, A. Klein, and R. K. Thauer.** 1992. H_2-forming methylenetetrahydromethanopterin dehydrogenase, a novel type of hydrogenase without iron-sulfur clusters in methanogenic archaea. Eur. J. Biochem. **208:**511–520.