Organization of threonine biosynthesis genes from the obligate methylotroph
Methylobacillus flagellatus

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The genes encoding aspartate kinase (ask), homoserine dehydrogenase (hom),
homoserine kinase (thrB) and threonine synthase (thrC) from the obligate
methylotroph Methylobacillus flagellatus were cloned. In maxicells hom and
thrC directed synthesis of 51 and 48 kDa polypeptides, respectively. The hom,
thrB and thrC genes and adjacent DNA areas were sequenced. Of the threonine
biosynthesis genes, only hom and thrC were tightly linked in the order
hom-thrC. The gene for thymidylate synthase (thyA) followed thrC and the
gene for aspartate aminotransferase (aspC) preceded hom. All four genes
(aspC-hom-thrC-thyA) were transcribed in the same direction. mRNA analysis
indicated that hom-thrC are apparently transcribed in one 7.5 kb transcript
in M. flagellatus. Promoter analysis showed the presence of a functional
promoter between aspC and hom. No functional promoter was found to be
associated with the DNA stretch between hom and thrC. The thrB gene
encoded an unusual type of homoserine kinase and was not linked to
other threonine biosynthesis genes.

Keywords: Methylobacillus flagellatus, threonine biosynthesis, homoserine
dehydrogenase, homoserine kinase, threonine synthase

INTRODUCTION

Methylobacillus flagellatus is an obligate methylotrophic bacterium able to grow only on methanol and
methylamine as sources of carbon and energy (Goverkhtina et al., 1987). This bacterium has a high
maximum growth rate (μ = 0.74) and a high methanol conversion coefficient (50 %, Baev et al., 1992). These
properties make M. flagellatus an ideal producer for the biotechnology industry. This bacterium has been shown
to have potential to be used for overexpression of heterologous proteins (Chistoserdov et al., 1987), pro-
duction of amino acids (threonine; Shilova et al., 1989) and vitamins (biotin; Serebriiski & Tsygankov, 1995).
Further development of M. flagellatus producing strains is hampered by the absence of basic knowledge about
the organization and functioning of its genes. Only a few genes have been cloned from M. flagellatus. These are
the gene for the RecA protein (Gomelsky et al., 1990) and the genes of biotin biosynthesis (Serebriiski
& Tsygankov, 1995). Our research is focused on the threonine biosynthesis processes in M. flagellatus.

The pathway of threonine biosynthesis has been shown to be similar in all micro-organisms (see Fig. 1).
However, the organization of the genes for threonine biosynthesis enzymes is different in different micro-
organisms. Some or all genes for threonine biosynthesis have been cloned from several Gram-positive bacteria,
several species of Corynebacterium (Mateos et al., 1987a, b; Kalinowski et al., 1991; Follette et al.,
1993; Malumbres et al., 1994:), several species of Mycobacterium (Cirillo et al., 1994), Lactobacillus lactis
(Le et al., 1996), Bacillus spp. (Bondaryk & Paulus,
Fig. 1. The pathway of threonine biosynthesis in M. flagellatus. Numbers refer to the following enzymes: 1, ASK; 2, ASD; 3, HDH; 4, HK; 5, TS. The biosynthetic pathways leading to lysine, methionine and isoleucine are shown by dashed lines. Dotted lines represent feedback inhibition of ASK and HDH (see Results and Discussion sections).

The pathway of threonine biosynthesis in M. flagellatus is shown in Fig. 1. Numbers refer to the following enzymes: 1, ASK; 2, ASD; 3, HDH; 4, HK; 5, TS. The biosynthetic pathways leading to lysine, methionine and isoleucine are shown by dashed lines. Dotted lines represent feedback inhibition of ASK and HDH (see Results and Discussion sections).

There have been only three reports in the literature about cloning genes involved in threonine biosynthesis from methylotrophic bacteria. The gene for ASD has been cloned from Thiobacillus versutus (Jagusztyn-Krynicki & Malashewsk-a-Keough, 1989), the gene for ASK has been cloned from a methylotrophic Bacillus sp. (Shendel & Flickinger, 1992) and the hom and thrC genes have been cloned from two species of Methylobacillus glycogenes (Motoyama et al., 1994). In this article, we report the organization of threonine biosynthesis genes from M. flagellatus which appears to be similar to that of P. aeruginosa and M. glycogenes. Preliminary results of this work have been presented previously (Marchenko & Tsygankov, 1992).

**METHODS**

**Bacterial strains, plasmids and growth conditions.** The bacterial strains and plasmids used in this work are shown in Table 1. All Escherichia coli strains were grown in Luria–Bertani medium in the presence of appropriate antibiotics as described by Maniatis et al. (1982). IPTG and X-Gal were added at 0.04 mg ml⁻¹. Methylobacillus flagellatus strains were grown in the minimal medium described previously (Govorukhina et al., 1987). The concentrations of ampicillin (Ap), rifampicin, streptomycin (Sm) and kanamycin (Km) for growing M. flagellatus strains were 1, 0.1, 0.15 and 0.15 mg ml⁻¹, respectively. In experiments with the promoter probe vector pAYC37, Sm was used in increasing concentrations up to 4 mg ml⁻¹. Methanol (2%, v/v) was used as carbon source.

**DNA manipulations.** Plasmid isolation, E. coli strain transformation, preparative isolation of the DNA fragments from agarose gels, restriction endonuclease digestion, ligation and blotting of ends with Klenow fragment or T4 DNA polymerase were carried out as described by Maniatis et al. (1982). Random primer labelling of DNA fragments was conducted as suggested by the manufacturer (NPO Ferment). Chromosomal DNA of the M. flagellatus strains was isolated using the procedure of Marmur (1961).

**Cloning of threonine biosynthesis genes.** A nearly complete (99.5%) EcoRI-generated gene library of M. flagellatus was constructed in the phasmid vector pSL5 (Yankovsky et al., 1989) in E. coli LE392. The pSL5 vector with 6–25 kb DNA inserts may exist either as a plasmid when the E. coli host is grown at 30°C or as phage particles when temperature is shifted to 42°C. Plasmids from the pSL5 library were transfected into E. coli strains with lesions in threonine biosynthesis genes as described by Yankovsky et al. (1989).

**RNA isolation.** Total RNA was isolated from E. coli TG1 and M. flagellatus as follows. Bacteria were grown in 10 ml minimal medium. Cells were collected by centrifugation, resuspended in 1 ml solution A (50 mM Tris–HCl, 50 mM EDTA, 5% SDS, pH 6.0) and incubated for 15 min at room temperature. Samples were transferred into a boiling water bath for 2 min, then quickly cooled down and centrifuged for 20 min in an Eppendorf microcentrifuge. The supernatant was extracted subsequently with acidic phenol (pH 5.0), phenol/chloroform and chloroform. RNA was precipitated with 2.5 vols cold ethanol in the presence of 0.2 M sodium acetate buffer (pH 5.0). The RNA precipitate was washed three times.
**Table 1.** Bacterial strains and plasmids used in this study

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<tr>
<th>Strain or plasmid</th>
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<td>F− thrB1 leuB6 thi-1 lacY1 supE44</td>
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<td>pPTS37</td>
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*Ap, ampicillin; Cm, chloramphenicol; Km, kanamycin; Rf, rifamycin; Sm, streptomycin; Su, sulfonamide; Tp, trimethoprim.
with cold 3 M sodium acetate (pH 5.0) to remove contaminating DNA.

DNA–DNA and RNA–DNA hybridizations. These were carried out in accordance with Maniatis et al. (1982). The temperature of hybridization (6 × SSC, 0.1% SDS) and washes (1 × SSC, 0.1% SDS) was 42 °C in the presence of 50% formamide for DNA–DNA hybridization and 65 °C without formamide for RNA–DNA hybridization (1 × SSC is 0.15 M sodium chloride, 0.015 M sodium citrate). The RNA Ladder RNA molecular size standard was obtained from Gibco-BRL.

DNA sequencing. DNA sequencing was performed by the dideoxy chain-termination method on both strands on an Applied Biosystems model 370A sequencer.

Computer analysis of DNA sequences. Computer analysis was carried out by using PCgene (Genofit) and the GCG software package (University of Wisconsin Genetic Computer Group). Multiple protein sequences were aligned using the CLUSTAL V program (Higgins et al., 1992) and alignments were refined visually. Phylogenetic analyses were conducted using the PROTPARS and NEIGHBOR programs from the PHYLIP 3.57c package (Felsenstein, 1989) and the PAUP 3.11 program (Swoford, 1991). Phylogenetic trees created by the three different programs were essentially identical.

Maxicell expression and electrophoresis of proteins. Plasmid-encoded proteins were labelled by the maxicell procedure described by Sancar et al. (1981) with the following modifications: strains bearing plasmids were E. coli AB2463 derivatives and these strains were irradiated with 40 J short wave UV light m⁻². SDS-PAGE was carried out according to Laemmli (1970). Separations were done in 14% (w/v) gels. Protein standards for SDS-PAGE were from New England Biolabs.

RESULTS

Cloning of ask, hom, thrB and thrC

The genes for ASK, HDH, HK and threanine synthase (TS) were cloned by complementation of E. coli strains GT14, Gif102, VL537 and GT25 (see Table 1), which have a lesion in threanine biosynthesis at the levels of ASK, HDH, HK and TS, respectively. These strains were infected with ßpSL5 phages (plasmids) from a M. flagellatus gene library and plated onto corresponding minimal media without threonine (VL537, Gif102 and GT25) or diaminopimelic acid (GT14).

Plasmids from GT14 clones, able to grow without diaminopimelic acid after infection with the ßpSL5 clone library, shared the same 10-4 kb EcoRI fragment (shown in Fig. 2). The ask gene was mapped within the 4 kb KpnI–Eco147I fragment. Southern blot hybridization was conducted to confirm that the 10-4 kb EcoRI fragment originates from M. flagellatus (data not shown). ASK activity was measured in crude extracts of M. flagellatus, E. coli GT14 and E. coli GT14(pASK6). No ASK activity was found in E. coli GT14 (Table 2). The higher specific ASK activities in E. coli GT14(pASK6) compared to these of M. flagellatus can be accounted for by the high copy number of the plasmid. The inhibition patterns of ASK activity in crude extracts from M. flagellatus and E. coli GT14(pASK6) were similar and did not resemble inhibition patterns of any of the three ASKs from E. coli. To verify whether the gene for ASK is linked to the gene for ASD, C600∆asd was transformed with pASK1A. None of the 100 analysed transformants was able to grow without diaminopimelic acid. Since the ask gene is located approximately in the middle of the 10-4 kb insert in pASK1A, the asd gene is not immediately adjacent to ask.

Plasmids which are able to complement E. coli VL537 carried two common 6·6 and 3·7 kb fragments (shown in the Fig. 2 as an insert in pTHRC1). pTHRC1 was not only able to complement the thrB mutation of strains VL334 and GT28 but it also conferred the ability to grow without methionine and threagine to E. coli Gif102. However, it did not complement the thrB mutation in E. coli AB2463. The genes for HDH and TS were more precisely localized within pTHRC1 by subcloning (Fig. 2). The smallest fragment able to

\[
\text{ask} \quad \text{thrA} \quad \text{thrC} \quad \text{hom} \quad \text{aspC} \quad \text{orfX} \quad \text{thrB} \quad \text{thrC} \quad \text{hom} \quad \text{aspC} \quad \text{orfX}
\]

**Fig. 2.** Restriction and genetic maps of plasmids carrying genes for ASK (pASK1A), HDH and TS (pTHRC1) and HK (pTHR5). Asterisks indicate that additional sites exist within inserts for these restriction endonucleases. The pUC19 portion of the plasmids is not shown.
complement the lesion in HDH in *E. coli* Gif102 was the 1·9 kb *PstI* fragment. The gene for TS (*thrC*) was mapped in the 1·6 kb *HindIII–Bpu1102I* fragment.

HDH from *M. flagellatus* is unusual in that it can use both NAD and NADP as electron acceptors (Table 3). Moreover, HDH activity with NAD is approximately five times higher than with NADP and it is not inhibited by threonine or methionine, or a combination of the two. The NADP-dependent activity of HDH is inhibited by methionine and threonine, and a combination of the two. NAD and NADP-dependent HDH activities expressed by pHOM12B in *E. coli* Gif102(pHOM12B) had similar ratio and inhibition patterns.

All plasmids complementing the *thrB* mutation of *E. coli* GT25 contained a common 4·3 kb *EcoRI* fragment (Fig. 2). The *thrB* gene was more precisely mapped within this fragment by subcloning. The smallest fragment of DNA containing the *thrB* gene was approximately 1·2 kb in size.

### Sequencing of hom, thr8 and thrC genes and adjacent areas

A 5·6 kb *PstI–ClaI* fragment from pTHRC1 containing the *thrC* and *hom* genes was sequenced. Five ORFs were identified (Fig. 2). A search in GenBank revealed that a polypeptide encoded by *orfX* (13463 Da, 122 aa) shares 28% similarity with the 13 kDa major membrane protein of *Francisella tularensis* (Sjoestedt et al., 1990) and 23% similarity with a hypothetical 12·7 kDa protein of *Rhodobacter capsulatus* (Beckman et al., 1992). The second ORF encoded a polypeptide (47790 kDa, 429 aa) which is similar to various aspartate aminotransferases from bacteria and yeasts (the highest identity of 65·8% was observed with AspC from *Haemophilus influenzae*;
The 1094 bp fragment from pTHR5 (Fig. 2), containing the entire gene for HK, was sequenced. Analysis of the sequence revealed that it encoded a polypeptide (35530 Da, 319 aa) which is 23% identical to one of the HKs described in P. aeruginosa (Clepet et al., 1992).

Complementation of the thrB mutation in M. flagellatus MFK11 by the cloned thrB gene

To prove that this gene is able to complement the thrB1 mutation in M. flagellatus MFK11, the thrB gene from pTHR5 was cloned in the broad-host-range vector pNGM130K and introduced into M. flagellatus MFK11 by conjugation. Out of 120 independent transconjugants analysed, all regained their ability to grow without supplemented threonine.

Expression of hom and thrC in E. coli maxicells

The recA strain E. coli AB2463 was used for maxicell expression of the cloned hom and thrC genes. Plasmids pTHRC1, pTHRC6 (contains the 3 kb HindIII–KpnI subfragment of pTHRC1) and pHOM12B (pUC19 with a 1.9 kb PstI fragment containing intact hom) were expressed in this strain. The results are shown in Fig. 2. pTHRC1 directs synthesis of four polypeptides of 52, 47, 38 and 28 kDa. The 28 kDa polypeptide apparently belongs to the mature form of β-lactamase (Fig. 3, lane D, pUC19). pTHRC6 directs synthesis of two polypeptides with molecular masses of 52 and 31 kDa. These correspond in molecular mass to TS (52131 Da) and thymidylate synthase (30131 Da), respectively. Although pTHRC1 and pTHRC6 directed synthesis of the HDH polypeptide at similar levels, synthesis of thymidylate synthase in cells containing pTHRC1 was very poor. pHOM12B directs synthesis of a polypeptide with a molecular mass of 47 kDa. Synthesis of the same polypeptide is directed by pTHRC1. This molecular mass estimate coincides with 46620 Da predicted for HDH from the sequence data. Sequence data also confirm that pHOM12B contains only one intact ORF, encoding HDH. The intensity of the 47 kDa band is much higher in the lane with the protein extract from cells containing pTHRC1 (Fig. 3). Apparently, the polypeptides of HDH and aspartate aminotransferase did not resolve well on the gel and this is consistent with their predicted molecular masses which are similar (46620 and 47790 Da, respectively). The 38 kDa polypeptide cannot be ascribed to any sequenced gene and it is apparently encoded by the DNA region downstream of the thyA gene.

Mapping of promoters and characterization of RNA transcripts for hom and thrC

The complementation analysis of E. coli Gif102 and GT28 has shown that the thrC and hom genes from M. flagellatus were expressed in E. coli under their own promoter(s), since expression of hom and thrC occurred independently of their orientation relative to the Plac promoter. The 426 bp Sau3A fragment, which includes the 5’ terminus of hom and 216 bp upstream, was cloned into the pAYC37 promoter probe vector (pPHD37). The HindIII–EcoRV fragment from pHOM12B which was
expected to contain putative promoter(s) for thrC was also inserted into pAYC37 (pPTS37). pPHD37, pPTS37, pAYC37 (negative control) and pAYC32 (positive control) were transferred into M. flagellatus MFK1 by conjugation and conferred levels of Sm resistance were determined for both E. coli and M. flagellatus. Both pPHD37 and pPTS37 provide Sm resistance in E. coli (100 and 150 µg Sm ml⁻¹, respectively), but only pPHD37 provided a substantial level of Sm resistance in M. flagellatus MFK1 (1-8 mg Sm ml⁻¹). The level of Sm resistance conferred to M. flagellatus by pPTS37 and pAYC37 was similar and low (30 µg Sm ml⁻¹). Thus, a functional M. flagellatus promoter exists only in front of the hom gene, indicating that the genes encoding HDH and TS are organized in one operon.

To further prove that the hom and thrC genes are co-transcribed, total mRNA was purified from M. flagellatus MFK1 and E. coli TG1 (the latter to be used as negative control) and hybridized with two probes: one was a 665 bp HincII internal fragment of the hom gene and the second was a 1002 bp Pael internal fragment of the thrC gene. The results of hybridization are shown in Fig. 4. Both probes hybridize with the same size mRNA transcript (~ 7.5 kb) confirming that HDH and TS in M. flagellatus are likely to comprise an operon.

**DISCUSSION**

**Organization of threonine biosynthesis genes in M. flagellatus**

The genes encoding enzymes involved in threonine biosynthesis, ASK (ask), HDH (hom), HK (thrB) and TS (thrC), were cloned from the methylo trophic bacterium M. flagellatus. Thus the only remaining gene required for threonine biosynthesis, which has not been cloned so far from this bacterium, is the gene for ASD. hom and thrC are linked in the chromosome of M. flagellatus and they are also linked to the aspC and thyA genes in the order aspC-hom-thrC-thyA. Our data indicate that hom and thrC are transcribed in M. flagellatus from a promoter located between aspC and hom as part of a single 7-5 kb mRNA transcript and, therefore, hom and thrC are organized in an operon. We expected that the hairpin structure identified between hom and thrC may serve as a transcriptional terminator. However, repeated Northern blot experiments clearly demonstrated the absence of an additional, smaller transcript which might contain hom mRNA only. Motoyama et al. (1994) have found that the two hom-thrC transcripts in M. glycogenes ATCC 21276 and ATCC 21371 are much smaller, only 1-4 and 2-4 kb in size, respectively. Despite the fact that the 1-4 kb transcript hybridized with both hom and thrC gene probes, it is not large enough to encode intact products of both genes.

Organization of the threonine biosynthesis genes similar to that of M. flagellatus was found in M. glycogenes (Motoyama et al., 1994) and P. aeruginosa (Clepet et al., 1992), although the cloning of aspC or thyA has not been reported in the two latter cases. The DNA sequence upstream of hom from M. glycogenes (Motoyama et al., 1994) shares substantial homology with DNA sequence upstream of hom from M. flagellatus. In addition, tfasta analysis (GCG package) indicated that AspC from M. flagellatus shares up to 90% identity with some, but not all, ORFs encoded by the upstream DNA sequence from M. glycogenes; however no ORF encoding the whole aspC gene could be found in the latter.

Three different arrangements of threonine biosynthesis genes have been found so far in bacteria (see Introduction). The organization of threonine biosynthesis genes found in M. flagellatus resembles that of non-enteric Gram-negative bacteria (Clepet et al., 1992; Motoyama et al., 1994). In these, hom and thrC and, at least in Methyllobacillus spp., aspC are clustered together. In all studied Gram-negative bacteria, including M. flagellatus, the genes for ASK and ASD are not linked to each other or to other threonine biosynthesis genes.

**Biochemical properties of ASK and HDH from M. flagellatus**

Based on the inhibition pattern of ASK from M. flagellatus it can be assumed that this bacterium encodes only one ASK which is inhibited in concert by threonine.
and lysine, but not methionine. This inhibition pattern has been found for ASKs from bacteria which do not have ASK isoenzymes, for example *C. glutamicum* (Nakayama et al., 1966) and *Pseudomonas* spp. (Ruban, 1986).

Hdh from *M. flagellatus* is a very unusual NAD(P)-dependent dehydrogenase. It has activity with both NAD and NADP, and the activity with NAD is approximately five times higher. The most striking feature of Hdh from *M. flagellatus* is that its NAD activity is not inhibited by methionine and threonine, whereas its NADP activity is inhibited by methionine and threonine. To our knowledge, no other Hdh with such properties has been described.

Analysis of the AspC, Hom, ThrC, ThyA and ThrB polypeptides from *M. flagellatus*

A GenBank search and consequent alignment indicated that the polypeptide most closely related to AspC from *M. flagellatus* is a putative AspC polypeptide from *H. influenzae*. It is interesting to note that AspC from *H. influenzae* is a much shorter polypeptide (251 aa) than that from *M. flagellatus* (437 aa). They share 66% identity along the first 243 aa. Aspartate aminotransferases from other sources have approximately the same number of amino acids as AspC from *M. flagellatus* but they share substantially lower levels of identity [from 14% for AspC from *Saccharomyces cerevisiae* (GenBank accession no. P47039) to 28% for AspC from *Bacillus* sp. (Sung et al., 1991)]. Alignment of several aspartate aminotransferases indicated that Lys240 in AspC from *M. flagellatus* is a putative pyridoxal-phosphate-binding amino acid. The sequence NGXS (where X is leucine, valine or phenylalanine) immediately upstream of this amino acid seems to be fairly conserved in sequenced bacterial AspC polypeptides.

The gene downstream of *aspC* encodes Hdh. Phylogenetic analysis of the Hom polypeptides confirms that they can be divided into three groups, with enterobacteria, *H. influenzae* and eukaryotes (carrot) in the first group, Gram-positive bacteria in the second group and non-enteric Gram-negative bacteria in the third group (data not shown). Results of phylogenetic analyses indicate that Hdhs with one activity (Hdh) are more closely related to each other than to Hdhs with two activities (ASK and Hdh). The NAD binding signature, GXGXXG, can be found in all HDhs, including *M. flagellatus* (aa 10–15). The Hom polypeptide from *M. glycorgenes* ATCC 21371 is the most closely related to Hom from *M. flagellatus*, sharing 78-2% identical amino acids. Surprisingly, Hdh from *M. glycorgenes* ATCC 21276 shares only 43-2 and 42-1% identity with *M. glycorgenes* ATCC 21371 and *M. flagellatus*, respectively.

The third gene in the threonine biosynthesis gene cluster from *M. flagellatus* is *thrC*. Phylogenetically, Ts from different sources are clustered into three groups: enterobacteria, eukaryotes and non-enteric bacteria. As expected, Ts from *M. glycorgenes* is the most closely related to ThrC from *M. glycorgenes* ATCC 21371; they share 91-2% identical amino acids. An alignment of Ts from different sources shows their high overall levels of similarity (not shown). The conserved region, 119-FKD-121, which binds the pyridoxal-phosphate co-enzyme of Ts (Parrot, 1986) is present in all Ts, including that from *M. flagellatus*.

The *thyA* gene is the third gene of the *hom-thrC* operon. It encodes a typical thymidylate synthase which has 68-2 and 67-8% identity with thymidylate synthases from *E. coli* (Belfort et al., 1983) and *B. subtilis* (Montorsi & Lorenzetti, 1993), respectively. Biosynthesis of pyrimidines and threonine requires the same metabolic ancestor, aspartate, and therefore it is reasonable to assume that some of the reactions of pyrimidines and threonine biosynthesis may be co-regulated. Based on our mRNA analysis, the 7-5 kb transcript, which encodes Hom, ThrC and ThyA, is large enough to encode three additional polypeptides with a mean size of ~ 50 kDa. Some of these polypeptides might be involved in earlier steps of pyrimidine biosynthesis.

The gene for HK is not clustered with other threonine biosynthesis genes in the *M. flagellatus* chromosome. A search of GenBank confirmed that this ORF is similar to one of the HKs described in *P. aeruginosa* and they are both dissimilar to HKs from all other bacteria. The ThrB polypeptides from *P. aeruginosa* and *M. flagellatus* share 23% identity, with highest overall similarity in the N-terminal regions of the polypeptides. It is likely that the unusual *thrB* gene found only in *M. flagellatus* and *P. aeruginosa* is the only HK-encoding gene in *M. flagellatus*. The *thrB* mutation in *M. flagellatus* MFK11 has been generated via chemical mutagenesis (Tsygankov et al., 1990) and it is highly unlikely that this lesion in HK is the result of a double mutation (in two HK genes).

In summary, we cloned and identified four out of five genes involved in threonine biosynthesis in *M. flagellatus* chromosome. These genes (except *hom* and *thrC*) are not linked to each other. Further analysis will be required to determine their relative location on the *M. flagellatus* chromosome and whether these genes are jointly regulated. Such knowledge can be used in the construction of threonine and other amino acid producers based on methylotrophic bacteria.

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