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# Cloning and Transcriptional Analysis of Two Threonine Biosynthetic Genes from *Lactococcus lactis* MG1614

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**Two genes, *hom* and *thrB*, involved in threonine biosynthesis in *Lactococcus lactis* MG1614, were cloned and sequenced. These genes, which encode homoserine dehydrogenase and homoserine kinase, were initially identified by the homology of their gene products with known homoserine dehydrogenases and homoserine kinases from other organisms. The identification was supported by construction of a mutant containing a deletion in *hom* and *thrB* that was unable to grow in a defined medium lacking threonine. Transcriptional analysis showed that the two genes were located in a bicistronic operon with the order 5′ *hom-thrB* 3′ and that transcription started 66 bp upstream of the translational start codon of the *hom* gene. A putative −10 promoter region (TATAAT) was located 6 bp upstream of the transcriptional start point, but no putative −35 region was identified. A DNA fragment covering 155 bp upstream of the *hom* translational start site was functional in pAK80, an *L. lactis* promoter probe vector. In addition, transcriptional studies showed no threonine-dependent regulation of *hom-thrB* transcription.**

The threonine biosynthetic pathway has been studied extensively in many prokaryotes, such as *Escherichia coli*, *Serratia marcescens*, *Corynebacterium glutamicum*, *Brevibacterium lactofermentum*, and *Pseudomonas aeruginosa* (see reference 23 for a review of studies of *E. coli*). In these organisms, threonine is synthesized from aspartate in five enzymatic reactions. The first two reactions common to diaminopimelate, lysine and methionine biosynthesis, are catalyzed by aspartokinase and aspartate semialdehyde dehydrogenase. The third reaction, catalyzed by homoserine dehydrogenase, is also part of the methionine biosynthetic pathway and leads to reduction of aspartate semialdehyde to homoserine, which is subsequently converted to threonine by homoserine kinase and threonine synthase in reactions 4 and 5, respectively.

Although the threonine biosynthetic pathways seem identical, the genetic organization, regulation of gene expression, and enzyme activity differ among the various organisms. In *E. coli* and *S. marcescens*, four of the five enzymes for threonine biosynthesis are encoded by the tricistronic *thrABC* operon (20, 31, 36). *thrA* encodes a bifunctional enzyme with aspartokinase and homoserine dehydrogenase activity. *thrB* and *thrC* encode the homoserine kinase and the threonine synthase, respectively, while *asd*, which encodes the aspartate semialdehyde dehydrogenase, is located elsewhere on the chromosome. Expression of the *thrABC* operon is regulated by threonine- and isoleucine-dependent transcriptional attenuation (10, 23). Furthermore, homoserine dehydrogenase activity is feedback inhibited by threonine (33).

In the corynebacteria *C. glutamicum* and *B. lactofermentum*, *hom* and *thrB* constitute an operon, whereas *thrC* is located at another chromosomal position (9, 13, 25, 27, 34). In *C. glutamicum*, expression of the *hom-thrB* operon is repressed by methionine (9) and the activity of homoserine dehydrogenase is feedback inhibited by threonine (29).

Yet another genetic organization has been found in *P. aeruginosa*, in which *hom* and *thrC* comprise a bicistronic operon, while *thrB* is located elsewhere on the chromosome (7). No regulation of threonine biosynthesis at the transcriptional level has been found in *P. aeruginosa*, but the homoserine dehydrogenase enzyme activity is feedback inhibited by threonine (7).

In this paper, we report the cloning, genetic organization, and transcriptional characterization of *hom* and *thrB* in *Lactococcus lactis* MG1614. Also, we describe the construction of a threonine auxotrophic mutant.

## MATERIALS AND METHODS

**Bacterial strains, media, and growth conditions.** *L. lactis* MG1614 (11) was grown at 30°C in rich M17 (Oxoid) medium containing 0.5% glucose or in a defined medium (SA) (17) containing 1% glucose. The concentration of L-threonine in the defined medium was 1.7 mM. *E. coli* DH5α (GIBCO-BRL) was grown at 37°C in LB (2) medium. *L. lactis* was plated on M17 agar (Oxoid) containing 0.5% glucose. The following concentrations of antibiotics were used: 100 μg of ampicillin per ml (*E. coli*), 250 μg of erythromycin per ml (*E. coli*), and 1 μg of erythromycin per ml (*L. lactis*). 5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) was used at 160 μg/ml in agar plates for *L. lactis*.

**β-Galactosidase determinations.** β-Galactosidase activity was determined on exponentially growing cultures as described by Miller (28), with modifications as specified by Israelsen et al. (16).

**DNA isolation and manipulation.** Chromosomal *L. lactis* DNA was prepared as described by Johansen and Kibenich (18). Plasmid DNA extractions were performed as previously described for *L. lactis* (32) and *E. coli* (4). DNA restriction and modification enzymes were purchased from GIBCO-BRL and used as recommended by the supplier. All DNA manipulations were performed as described by Maniatis et al. (26). DNA was electroporated into competent *L. lactis* cells grown in glycine (15) and into *E. coli* as described by Hanahan (14).

**Cloning of *hom* and *thrB* from *L. lactis* MG1614 by using Tn*917*-LTV1 and inverse PCR.** Plasmid p243 (16) was digested with *Eco*RI and *Cla*I, and a 1.8-kb chromosomal *L. lactis* fragment was inserted into pGEM 7Zf(+) (Promega), resulting in pSMA231 (Fig. 1). This fragment contained the entire *thrB* gene and an incomplete *hom* gene. Two inverse PCRs (30) were performed to clone the 5′ end of *hom*. Chromosomal DNA from MG1614 was digested with *Ssp*I, phenol-chloroform extracted, and ethanol precipitated. The DNA (60 ng) was ligated in a relatively large volume (20 μl) with 1 U of T4 DNA ligase to favor the formation of monomeric circles. A 5-μl sample was PCR amplified in 100 μl, using a GeneAmp DNA amplification reagent kit from Perkin-Elmer Cetus, primers BA17 (5′ GGT CAA AAA CCC ACA GCT ACG AG; positions 1062 to

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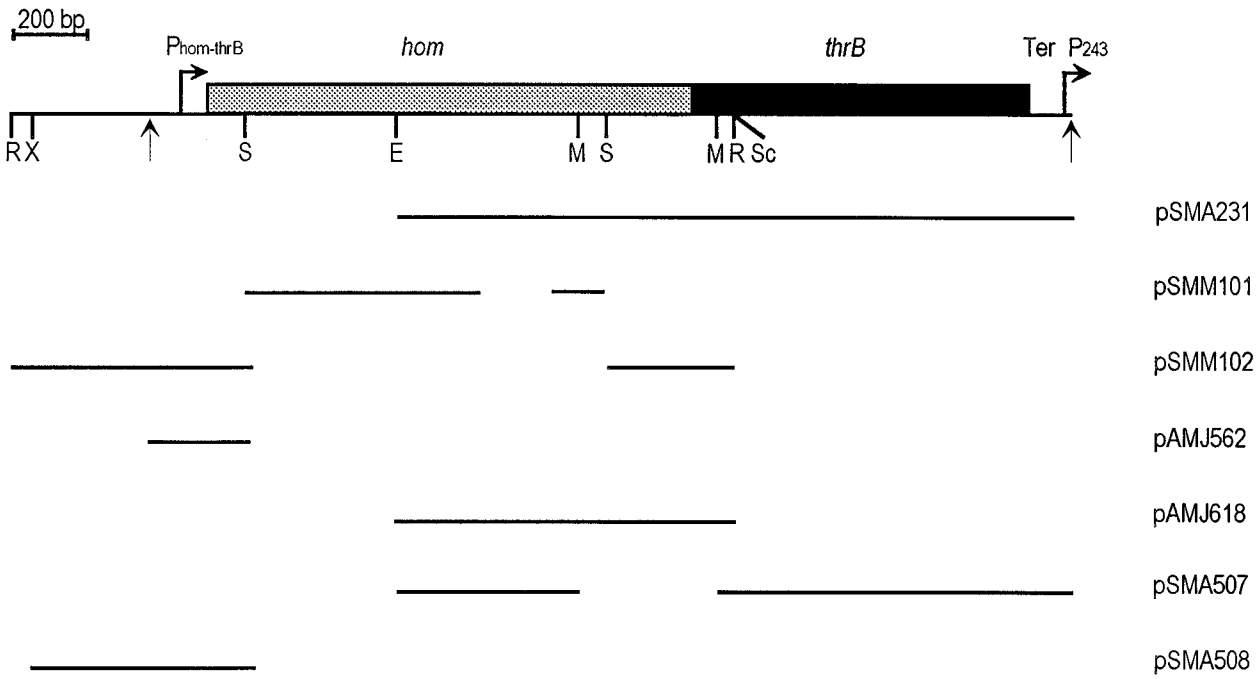


FIG. 1. Physical map of the *hom-thrB* operon from *L. lactis* MG1614. $P_{hom-thrB}$ and $P_{243}$ indicate the promoter transcribing the *hom-thrB* operon and the downstream $P_{243}$ promoter, respectively. Ter indicate the putative Rho-independent transcription terminator. The region between the vertical arrows corresponds to the DNA sequence presented in Fig. 2. The restriction map shows only relevant sites used in the DNA constructions described in Materials and Methods. E, *Eco*RI; M, *Mun*I; R, *Rsa*I; S, *Ssp*I; Sc, *Sca*I; X, *Xho*I. The solid lines below the physical map represent the lactococcal fragments of DNA present in the various plasmids.

1084 in Fig. 2) and BA24 (5′ CCA GTC AAC TTT AAA ACA TAA CC; positions 907 to 885 in Fig. 2), and reaction conditions as recommended by the supplier. Reaction mixtures were kept for 1 min at 94°C, 1 min at 53°C, and 2 min at 72°C for 40 cycles followed by 10 min at 72°C. The resulting 0.8-kb fragment was cloned into pT7Blue(R) (Novagen), resulting in plasmid pSMM101 (Fig. 1). Sequence analysis showed that pSMM101 contained only part of the *hom* gene. Primers SBINV1 (5′ GAA ATG ACG ATT TCT TCA TCC; positions 271 to 251 in Fig. 2) and BA28 (5′ TAT TTT TCA GTT GAG ACA CCT G; positions 1206 to 1227 in Fig. 2) were used in a second inverse PCR using chromosomal DNA from MG1614 digested with *Rsa*I. A 1.1-kb PCR fragment was cloned into pT7Blue(R), resulting in pSMM102 (Fig. 1). Sequence analysis revealed that pSMM102 contained the remaining part of the *hom* gene.

**Construction of integration vector pSMA500 and its derivatives.** pSMA500 was made by inserting a 4.0-kb *Hin*dIII-*Sal*I fragment that contains the polylinker and the promoterless β-galactosidase reporter genes from the promoter cloning vector pAK80 (16) into pVA891 (24). pSMA244 was constructed by deleting an internal *Mun*I fragment covering 302 bp of the 3′ end of the *hom* gene and 52 bp of the 5′ end of the *thrB* gene from pSMA231. pSMA507 (Fig. 1) was made by moving a 1.5-kb lactococcal *Xho*I-*Bam*HI fragment, containing a 3′-truncated *hom* gene and a 5′-truncated *thrB* gene, from pSMA244 into pSMA500. pSMA243 was constructed by moving a lactococcal 1.1-kb *Kpn*I-*Hin*dIII fragment from pSMM102 into pGEM 7Zf(+). The lactococcal DNA in pSMA243 contains an internal *Xho*I site that was used to move a 0.6-kb *Xho*I-*Bam*HI fragment from pSMA243 into pSMA500, resulting in pSMA508 (Fig. 1).

**Construction of pAMJ562 and pAMJ618.** By using primers SB XHOI (5′ GGC CGC TCG AGC TGA TTA ATC TGT CAG; positions 1 to 16 in Fig. 2) and SB INV1 BAMHI (5′ TAG TAG GAT CCG AAA TGA CGA TTT CTT CAT CC; positions 271 to 251 in Fig. 2), a 293-bp fragment was PCR amplified from the *L. lactis* MG1614 chromosome and digested with *Xho*I and *Bam*HI. The resulting fragment, which covers 155 bp upstream of and 116 bp downstream of the *hom* translational start codon, was inserted into the promoter probe vector pAK80, resulting in pAMJ562 (Fig. 1). A 0.9-kb *Eco*RI-*Sca*I fragment covering 815 bp of the 3′ end of the *hom* gene and 92 bp of the 5′ end of the *thrB* gene was inserted into pAK80, resulting in pAMJ618 (Fig. 1).

A physical map of the *hom-thrB* operon and relevant plasmids are shown in Fig. 1.

**DNA sequencing and sequence analysis.** Double-stranded plasmid DNA was sequenced by the dideoxy-chain termination method (35), using a Sequenase 2.0 kit from United States Biochemical. All DNA primers used for PCR, primer extension, and sequencing were synthesized by DNA Technology ApS, Science Park Aarhus, Denmark. DNA and amino acid sequences were analyzed by using the GCG program package from the University of Wisconsin Genetics Computer Group. Protein homology searches in the Swiss Protein database (release 31.0) were carried out with the FASTA program.

**RNA isolation and Northern (RNA) hybridization.** *L. lactis* MG1614 was grown exponentially in a defined medium with or without 1.7 mM L-threonine. At an optical density at 600 nm of 0.5, cells from 40 ml of culture were cooled in liquid nitrogen and harvested, and total RNA was isolated by the method of Arnau et al. (1). The RNA pellet was resuspended in 30 μl of diethyl pyrocarbonate-treated water at a concentration of 5 to 10 μg/μl. Twenty micrograms of total RNA from each preparation was denatured in formamide loading buffer and separated on a 1.3% (wt/vol) agarose–0.66 M formaldehyde gel. The 0.24- to 9.5-kb RNA ladder from GIBCO-BRL was used as the marker. Following electrophoresis, the gel was soaked in 1× SSC (0.15 M sodium chloride plus 0.015 M sodium citrate) containing 0.05 M NaOH for 10 min and washed twice in 10× SSC for 15 min. The RNA was transferred to a GeneScreen Plus hybridization transfer membrane (DuPont) by capillary blotting overnight and fixed by baking for 2 h at 80°C. The membrane was prehybridized for 4 h at 65°C in 0.5 M sodium phosphate (pH 7.2) that contained 7% sodium dodecyl sulfate (SDS) and hybridized for 20 h at 65°C in the same buffer. The hybridization probe was randomly labeled with 50 μCi of [α-$^{32}$P]dCTP (Amersham). After hybridization, the membrane was washed three times for 15 min each at 65°C in 20 mM sodium phosphate (pH 7.2) containing 1% SDS. Finally, the membrane was dried and X-ray film was exposed for 20 h.

**Primer extension.** The 5′ end of the mRNA was determined by the 5′ AmpliFINDER RACE (rapid amplification of cDNA ends) kit from Clonetech Laboratories, Inc. The procedure recommended by the supplier was followed, with minor modifications. Fourteen micrograms of total RNA isolated from cells grown in a defined medium without threonine was used as the cDNA template. The SB thrA primer (5′ CCG TCT ACA TCA TTT GTT GGG TCG GAT TCA GCA TAA CCA AGT TCT TGA GCT TTA GCC AAG G; positions 754 to 694 bp in Fig. 2) was used to prime cDNA synthesis. After cDNA synthesis, the RNA template was hydrolyzed by NaOH, the sample was neutralized, and excess primer was removed by differential binding of the cDNA to a glass matrix. The cDNA was ethanol precipitated, and a specially designed single-stranded anchor oligonucleotide was ligated to the 3′ end of the cDNA. The cDNA was subsequently amplified by PCR, using a primer complementary to the anchor oligonucleotide and primer SBINV1, which is complementary to *hom*. The resulting approximately 230-bp fragment was inserted into phagemid pBluescript II KS (Stratagene) digested with *Eco*RV, and the 5′-terminal end of the *hom-thrB* mRNA was determined by DNA sequencing.

**Nucleotide sequence accession number.** The nucleotide sequence data reported here will appear in the EMBL nucleotide sequence database under accession number X96988.

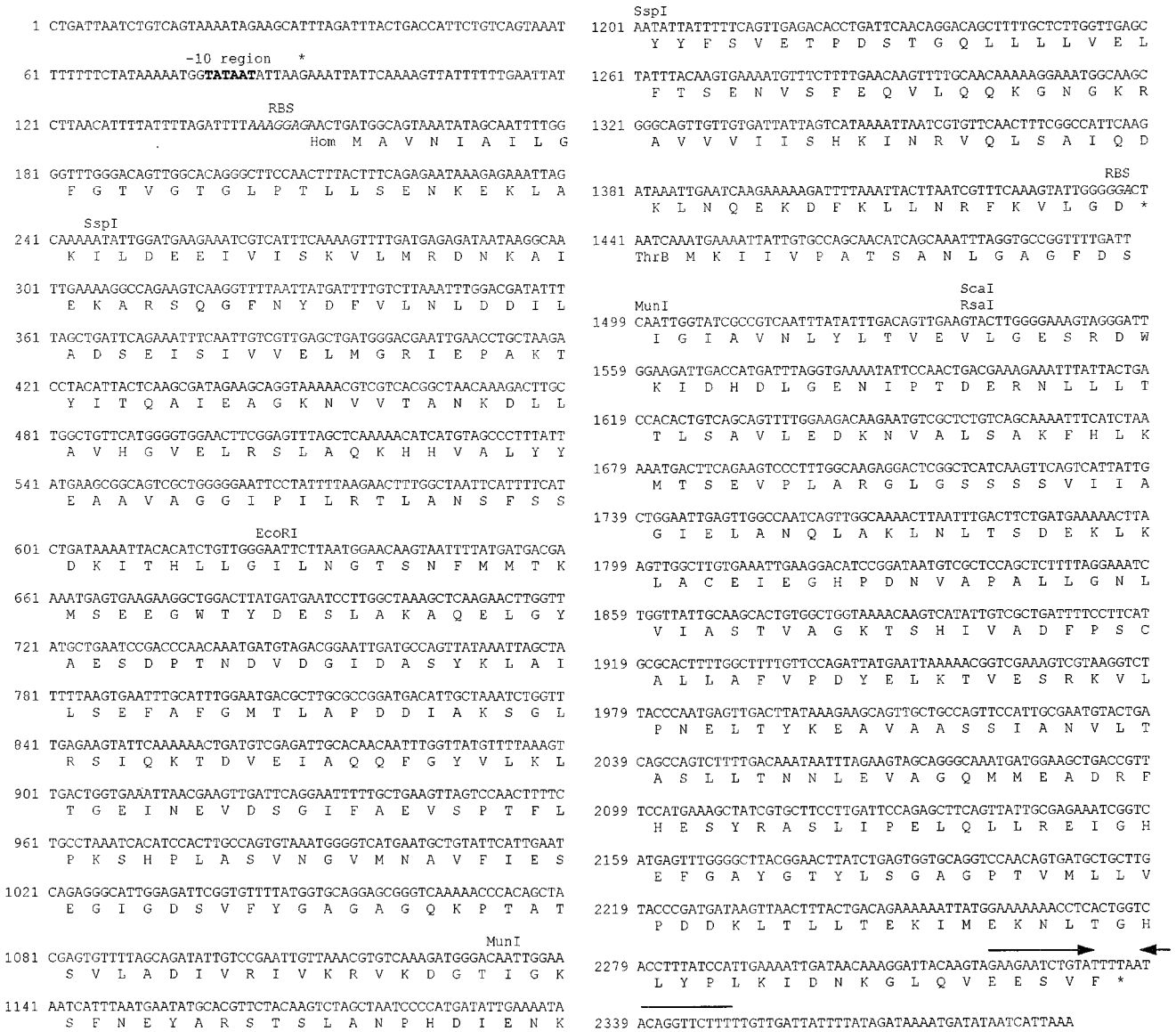


FIG. 2. Nucleotide and deduced amino acid sequences of the *L. lactis hom-thrB* region. Numbers at the left refer to nucleotides. Only restriction sites used in experiments are shown. The putative −10 region is boldfaced, and putative ribosome-binding sites (RBS) are italicized. The transcription start point is indicated by an asterisk above the nucleotide sequence. The putative Rho-independent transcription terminator is indicated by horizontal arrows above the nucleotide sequence. Asterisks beneath the nucleotide sequence indicate translational stop codons.

## RESULTS

**Two open reading frames in *L. lactis* are homologous to *hom* and *thrB* from *Bacillus subtilis*.** While cloning and analyzing chromosomal promoters from a collection of *L. lactis* MG1614 Tn*917*-LTV1 integrants, we identified two open reading frames (part of ORF1 and ORF2) upstream of the lactococcal promoter $P_{243}$ in plasmid p243 (16). Since part of ORF1 was missing in p243, inverse PCR was used to clone its 5′ end. The nucleotide sequence is presented in Fig. 2. Homology analysis showed that ORF1 (428 amino acids) is 38, 37, and 30% identical to the homoserine dehydrogenases of *B. subtilis*, *C. glutamicum*, and *P. aeruginosa*, respectively, and that ORF2 (296 amino acids) is 36, 30, and 30% identical to the homoserine kinases of *B. subtilis*, *C. glutamicum*, and *B. lactofermentum*, respectively. The identities indicate that ORF1 is the *hom* gene and ORF2 is the *thrB* gene of *L. lactis* MG1614. No open reading frames were found in the 155 bp preceding *hom*. A putative Rho-independent transcriptional terminator was found between *thrB* and the promoter $P_{243}$. The $\Delta G$[25°C] value of this terminator was −11.2 kcal (1 kcal = 4.184 kJ)/mol (37). Both *hom* and *thrB* are preceded by putative ribosome-binding sites complementary to the 3′ end of the lactococcal 16S rRNA (5), with $\Delta G°$ values of −14 and −7.2 kcal/mol, respectively.

***hom* and *thrB* form an operon transcribed from a promoter located upstream of *hom*.** To determine the length of the transcript(s) and the operon structure, Northern blot analysis was performed. Total RNA was extracted from *L. lactis* MG1614 grown exponentially in a defined medium lacking threonine and hybridized with a 1,700-bp *Eco*RI-*Cla*I fragment covering 0.8 kb of the 3′ end of *hom* and the entire *thrB*. Northern analysis showed one major 2.3-kb transcript (Fig. 3),

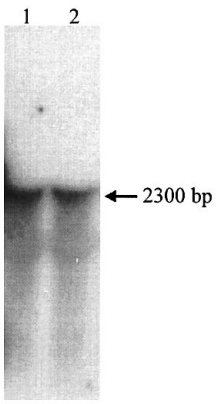


FIG. 3. Northern blot analysis of the *hom-thrB* operon. Total RNA was isolated from *L. lactis* grown in a defined medium with (lane 1) or without (lane 2) threonine. The 2,300-bp transcript is indicated.

as expected from the DNA sequence if *hom* and *thrB* were cotranscribed and terminated at the putative *thrB* terminator.

Two DNA fragments containing 155 bp upstream of *hom* and the region covering 815 bp of the 3′ end of *hom* and 92 bp of the 5′ end of *thrB*, respectively, were inserted into the promoter probe vector pAK80. The resulting plasmids, pAMJ562 and pAMJ618, were introduced into *L. lactis* MG1614, and the β-galactosidase activity was determined. Plasmid pAMJ562 resulted in 18 Miller units, demonstrating the existence of a functional promoter on the inserted DNA fragment, while pAMJ618 resulted in <0.1 Miller units, corresponding to pAK80 without an inserted fragment. These results showed that *hom* and *thrB* are transcribed from the same promoter located upstream of *hom.*

Primer extension was performed to pinpoint the transcription start site. The start point was mapped to a G 66 bp upstream of the ATG translational start codon of *hom*. The sequence TATAAT was found 6 bp upstream of the 5′ end of the *hom-thrB* mRNA. This putative −10 region was immediately preceded by the sequence TGN, which is often found in lactococcal promoters (8, 39). No putative −35 region was identified 16 to 20 bp upstream of the −10 region.

**Construction of a threonine auxotrophic mutant.** To construct a threonine auxotrophic mutant (Fig. 4) by gene replacement (22), a new integration vector, pSMA500, was developed from the promoter cloning vector pAK80. It contains an erythromycin resistance gene, which is expressed in both *E. coli* and *L. lactis*, an *E. coli* origin of replication, and promoterless β-galactosidase reporter genes preceded by a polylinker region. Because pSMA500 cannot replicate in *L. lactis*, erythromycin resistance will be obtained only if the plasmid integrates into the lactococcal genome. pSMA507, derived from pSMA500, contains the 3′ end of *hom* and the entire *thrB* and importantly a deletion covering the 302 distal bp of *hom* and the proximal 52 bp of *thrB*. This plasmid was introduced into *L. lactis* MG1614, and then transformants were plated on GM17 plates containing erythromycin and X-Gal. Ten randomly picked blue transformants lacked autonomously replicating plasmid DNA, indicating chromosomal integration of pSMA507. The integration most likely occurred by a single crossover between homologous DNA on the plasmid and the chromosome. To obtain a chromosomal deletion of the *hom*

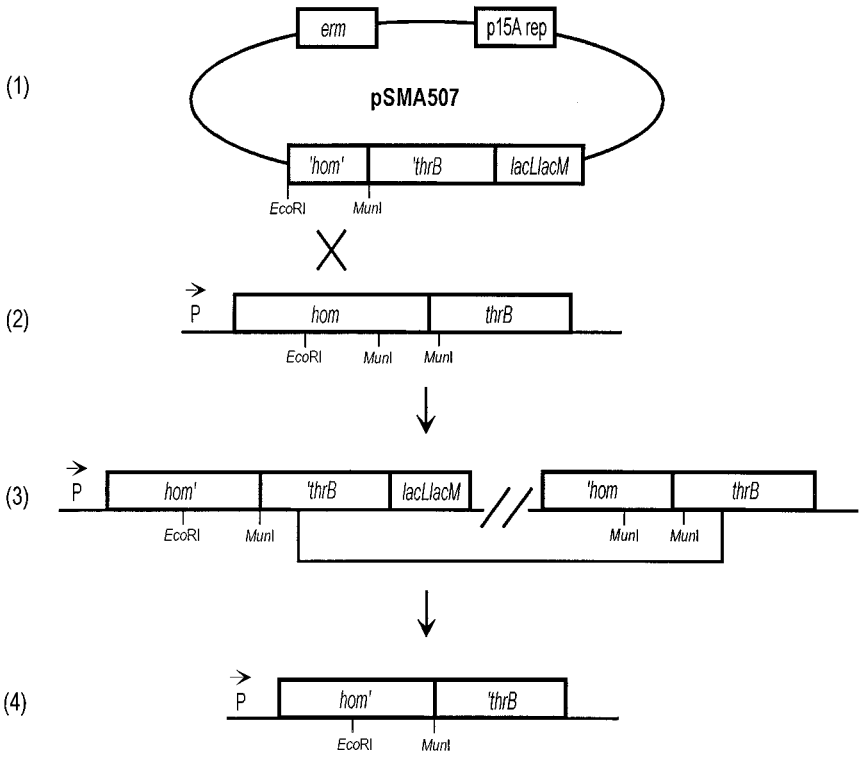


FIG. 4. Construction of an auxotroph threonine mutant. (1) Plasmid pSMA507 contains *hom* truncated at both ends (*'hom'*). *'thrB* indicates a 5′ truncation of *thrB*. The *E. coli* p15A replicon (p15A rep) and the erythromycin resistance marker (*erm*) are also indicated. (2) Structure of the wild-type *hom-thrB* operon. (3) The result of a Campbell-like integration of plasmid pSMA507 into the lactococcal genome. Only part of the intervening plasmid sequence is shown. (4) Subsequent recombination between the homologous DNA sequences of *'thrB* and *thrB* results in the formation of a *hom-thrB* deletion mutant. The figure is not drawn to scale.

and *thrB* genes, a restreaked integrant was grown in GM17 medium without erythromycin for 75 generations. Cells were plated on GM17 agar containing X-Gal and screened for loss of β-galactosidase activity. The lack of β-galactosidase activity was expected to be the consequence of a second crossover event, resulting either in a wild-type chromosome or a chromosome containing a deletion in both *hom* and *thrB*. Chromosomal DNA from six white recombinant strains was analyzed by PCR, using two primers located outside the deletion. A 768-bp PCR fragment should be produced from a wild-type chromosomal template, whereas a template containing the expected deletion would result in a 390-bp fragment. Four of the six strains contained the expected deletion (data not shown) and grew in a defined medium containing threonine but not in the same medium lacking threonine. The positive control, *L. lactis* MG1614, grew in the medium lacking threonine. This result clearly demonstrated that *hom* and/or *thrB* are essential for threonine biosynthesis in *L. lactis* MG1614.

**Transcription of *hom-thrB* in *L. lactis* is unaffected by threonine in the growth medium.** To investigate threonine's influence on chromosomal *hom-thrB* transcription, we constructed plasmid pSMA508, which contains about 500 bp upstream of and 116 bp downstream of the *hom* translational start codon in transcriptional fusion with the β-galactosidase reporter genes of pSMA500. Plasmid pSMA508 was introduced into *L. lactis* MG1614, and transformants were selected on GM17 plates containing X-Gal and erythromycin. Ten randomly picked blue transformants lacked autonomously replicating plasmid DNA, indicating a single crossover between homologous DNA on the plasmid and the chromosome. Southern hybridization showed that the copy number of pSMA508 in the chromosome varied from one to several among the analyzed transformants. Strain SMA508A, which contained a single copy of chromosomally integrated pSMA508, was grown exponentially in a defined medium containing threonine and then shifted (at an optical density at 600 nm of 0.5) to a defined medium either containing or lacking threonine. After the shift, β-galactosidase activities were 2.4 ± 0.6 Miller units in the defined medium lacking threonine and 2.8 ± 0.3 Miller units in the defined medium containing threonine. These enzyme activities were unaffected by the number of generations the culture was grown after the shift. The results showed that threonine per se at the most affects transcription of the *hom-thrB* operon marginally. Northern analysis of total RNA extracted from *L. lactis* MG1614 grown with or without threonine showed no difference in the intensity of the 2.3-kb band (Fig. 3). This finding supports the conclusion that threonine has no effect on regulation of *hom-thrB* transcription.

## DISCUSSION

In this report, we describe the identification and analysis of the *hom* and *thrB* genes encoding homoserine dehydrogenase and homoserine kinase from the threonine prototroph *L. lactis* MG1614. These genes are flanked by a promoter upstream of *hom* and by a putative Rho-independent transcriptional terminator downstream of *thrB*. Transcriptional studies showed that the genes are organized in an operon with the order 5′ *hom-thrB* 3′. The *thrC* gene, encoding threonine synthase, is not included in the operon. This gene organization is similar to that of the two corynebacteria *C. glutamicum* and *B. lactofermentum* (9, 27, 34). Using the promoter probe vector pAK80, we found that a promoter is present within the 155 bp upstream of the translational start site of *hom*. No evidence for *thrB* transcription independent of *hom* transcription was found. This conclusion is based on the presence of a single *hom-thrB* transcript in the Northern experiment and on the absence of promoter activity on the DNA segment cloned in pAMJ618. Even though *hom-thrB* is organized similarly in *L. lactis* and *B. lactofermentum*, a promoter has been identified upstream of *thrB* in *B. lactofermentum* (27).

Using primer extension analysis, we mapped the start site for *hom-thrB* transcription to a G 66 bp upstream of the ATG start codon of *hom*. Although a putative −10 sequence was found 6 bp upstream of the transcriptional start point, no putative −35 sequence could be identified 16 to 20 bp further upstream. Other functional *Lactococcus* promoters devoided of a properly located −35 region have been described by van Asseldonk et al. (38) and by Bidnenko et al. (3). Several such promoters which are recognized by the major sigma factor $\sigma^{70}$ have also been identified in *E. coli*. They all have an additional TGN motif (extended −10 region) located immediately upstream of the −10 region (19). In *E. coli*, the transcription of −35 independent promoters often requires the binding of activator proteins (21). The *Lactococcus* phage bIL66 promoter contains this extended −10 consensus sequence (3). Analysis of the DNA sequence located upstream the −10 region of *hom-thrB* is also in accordance with this extended −10 consensus sequence. In addition, an AT-rich region (87%) is present upstream of the −10 region, but we do not know if this region plays a role in the efficiency of transcription initiation in *L. lactis*. However, van Rooijen et al. (40) described the importance of an AT-rich region upstream of the −35 region and showed that this region contributed more than 10-fold to the efficiency of transcription initiation in *L. lactis.*

Transcription of the *hom-thrB* operon showed no threonine-dependent regulation. This was shown both by using transcriptional gene fusions and by Northern analysis of total RNA extracted from cultures grown in a defined medium with or without threonine. Even growth in rich GM17 medium did not repress *hom-thrB* transcription (data not shown). These results were unexpected because three other amino acid biosynthetic gene clusters from *L. lactis* are regulated at the transcriptional level. The *leu-ilv* operons in *L. lactis* NCDO2118 and *L. lactis* IL1403 are regulated by transcriptional attenuation involving a leader peptide (6, 12). The *his* operons in the same strains and the *trp* operon of *L. lactis* IL1403 seem to be regulated by transcription antitermination whereby readthrough of the structural genes relies on the conformational structure of the preceding leader mRNA (6). A terminator or an antiterminator is formed, depending on the ability of a tRNA molecule to interact with a so-called T-box sequence located in the leader mRNA. Sequence analysis of the 66-bp untranslated *hom-thrB* leader from *L. lactis* MG1614 did not show any leader peptide, terminator or antiterminator, or T-box-like elements, supporting the observed lack of regulation at the transcriptional level. Limited ability in threonine uptake by *L. lactis* could explain this observation. However, the specific growth rate of *L. lactis* MG1614 in the defined medium containing threonine was about 50% higher than that in the same medium lacking threonine, indicating that threonine is transported into the cell (data not shown). We cannot exclude the possibility that removal of amino acids other than threonine will affect the transcription of the *hom-thrB* operon in *L. lactis*. The observed transcriptional level could be a repressed level, and induction could possibly occur if threonine in combination with other amino acids were removed.

Although the *hom-thrB* genes are not regulated by threonine at the transcriptional level, expression of the genes could be translationally regulated or the enzymes could be feedback inhibited by threonine or other amino acids. The lack of transcriptional regulation of threonine biosynthetic genes has pre-

viously been observed in *P. aeruginosa*, in which the only regulation consists of feedback inhibition of homoserine dehydrogenase by threonine (7). A similar regulation of enzyme activity may take place in *L. lactis*. Regulation at the translational level is also conceivable; homoserine dehydrogenase and homoserine kinase might be needed in different stoichiometric ratios. This hypothesis is supported by different free energies in the respective ribosome-binding sites preceding *hom* and *thrB*, suggesting a control in the efficiency of initiation of translation.

Presently, we are constructing an *L. lactis* strain lacking the *hom-thrB* operon and vectors containing this operon as an alternative to antibiotic selection for maintaining plasmids in *L. lactis.*

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