

## Histidine Biosynthesis Genes in *Lactococcus lactis* subsp. *lactis*

CHRISTINE DELORME,\* S. DUSKO EHRLICH, AND PIERRE RENAULT

Laboratoire de Génétique Microbienne, Institut National de la Recherche Agronomique,  
78352 Jouy-en-Josas Cedex, France

Received 21 April 1992/Accepted 12 August 1992

The genes of *Lactococcus lactis* subsp. *lactis* involved in histidine biosynthesis were cloned and characterized by complementation of *Escherichia coli* and *Bacillus subtilis* mutants and DNA sequencing. Complementation of *E. coli* *hisA*, *hisB*, *hisC*, *hisD*, *hisF*, *hisG*, and *hisIE* genes and the *B. subtilis* *hisH* gene (the *E. coli* *hisC* equivalent) allowed localization of the corresponding lactococcal genes. Nucleotide sequence analysis of the 11.5-kb lactococcal region revealed 14 open reading frames (ORFs), 12 of which might form an operon. The putative operon includes eight ORFs which encode proteins homologous to enzymes involved in histidine biosynthesis. The operon also contains (i) an ORF encoding a protein homologous to the histidyl-tRNA synthetases but lacking a motif implicated in synthetase activity, which suggests that it has a role different from tRNA aminoacylation, and (ii) an ORF encoding a protein that is homologous to the 3'-aminoglycoside phosphotransferases but does not confer antibiotic resistance. The remaining ORFs specify products which have no homology with proteins in the EMBL and GenBank data bases.

The histidine pathway, which requires 11 enzymatic reactions, has been extensively analyzed in *Escherichia coli* and *Salmonella typhimurium* (10, 46). The enzymes are encoded by only eight genes in these bacteria, since the products of the *hisD*, *hisB*, and *hisIE* genes are bifunctional. The eight genes are organized in a single operon, which is regulated by attenuation (3, 8, 40). The organization of *his* genes has not been fully determined in any other organism. In *Staphylococcus aureus*, six of the genes (*hisE*, *-A*, *-B*, *-C*, *-D*, and *-G*) are clustered, whereas in *Streptomyces coelicolor*, five (and possibly six) genes (*hisD*, *-C*, *-B*, *-H*, *-A*, and possibly *-F*) form an operon and two (*hisIE* and *hisB*) are independent (30, 36). In *Bacillus subtilis*, the genes map in two locations, one grouping seven genes (*hisA*, *-B*, *-D*, *-F*, *-G*, *-C*, and *-IE*) and the other containing a single gene (*hisH*, corresponding to *hisC* in *E. coli*) (11, 18, 25, 37). The *hisI* gene from a methanogenic archaeobacterium is separated from the other *his* genes (6). Some of the *his* genes in several eukaryotes have been characterized (29, 33). In *Saccharomyces cerevisiae*, the *HIS4* gene encodes a multifunctional enzyme that catalyzes four steps in the biosynthetic pathway. It contains three domains homologous to HisI, HisE, and HisD of *E. coli* (8). A similar enzyme is found in *Candida albicans* (1).

In this paper, we report the organization of a cluster of *Lactococcus lactis* subsp. *lactis* genes, encoding eight of the nine histidine biosynthesis enzymes as well as six other genes of unknown function. Two of the six other genes specify proteins homologous to the 3'-aminoglycoside phosphotransferases (Apha-3') and histidyl-tRNA synthetases, respectively, but do not appear to possess the corresponding functions. Products of the four other genes are not homologous to sequences deposited in data banks. Similar studies of the *L. lactis* subsp. *lactis* genes for tryptophan and branched-chain amino acid biosynthesis are reported in the accompanying papers (4, 23).

### MATERIALS AND METHODS

**Bacterial strains, plasmids, and media.** The bacterial strains and plasmids used are described in Table 1. *B. subtilis* IL4013 was constructed by replacing the *hisH* gene of the restrictionless strain MT119 with the *hisH* gene lacking an internal 339-bp segment, carried on plasmid pHHD (25). Media and growth conditions are described in an accompanying report (4).

**Molecular cloning and DNA manipulations.** Molecular cloning and DNA manipulations are described in an accompanying report (4). The 3' end of the *his* cluster was isolated by inverse polymerase chain reaction, using oligonucleotides CATCTGCACACTGTTCTTCATAT and TCAATTCCATTAACCTTTGGTGG, which are complementary to nucleotides 8757 to 8780 and 8863 to 8886, respectively, of the reported sequence. Annealing (1 min at 50°C) and elongation (3 min at 70°C) were carried out on a Perkin Elmer Cetus Gene Amp PCR System 9600.

**DNA sequence analysis.** Nested deletions were produced by action of DNase I on pBluescript (pBS) plasmids containing the fragments to be sequenced. DNA sequence analysis is described in reference 4. Rearrangements were observed on certain clones during production of single-stranded DNA. These clones were sequenced by the standard manual dideoxynucleotide technique with the Sequenase kit (Stratagene). The reported sequence was determined on both strands.

### RESULTS

**Cloning of the *his* region.** Total DNA prepared from *L. lactis* subsp. *lactis* NCDO2118 was partially digested with endonuclease *Sau3A* to produce fragments with an average size of 10 kb. Twenty micrograms of fragments was ligated to 10 µg of pIL253 DNA cleaved with *Bam*HI at a final DNA concentration of 500 µg/ml and used to transform *B. subtilis* IL4013, which lacks histidinol phosphate aminotransferase activity, to histidine prototrophy and Em<sup>r</sup>. Seventy-two His<sup>+</sup> and Em<sup>r</sup> transformants were obtained, and the plasmid contents of two clones were analyzed. Two different plasmids, named pIL378 and pIL381, carrying inserts of 9 and 3.6 kb, respectively, were detected.

\* Corresponding author.

TABLE 1. Strains and plasmids

Strain or plasmid	Characteristics	Reference(s) or source
<b>Strains</b>		
<i>L. lactis</i> subsp. <i>lactis</i>		
IL1403		13
NCDO2118	Isolated from frozen peas	National Collection of Dairy Organisms
<i>B. subtilis</i>		
MT119	<i>leuB6 trpC2 r<sup>-</sup></i>	Bacillus Genetic Stock Center
IL4013	<i>hisH trpC2 leuB6 r<sup>-</sup> m<sup>-</sup></i>	This work
BC399	<i>hisA1 argC4 metD1 phe-1</i>	V. Sgaramella
<i>E. coli</i>		
TG1	<i>supE thi Δ(lac-proAB) hsdD5(F<sup>+</sup> traD36 proAB lacI ZΔM15)</i>	22a
Hfr G6	<i>hisA323 λ<sup>-</sup></i>	<i>E. coli</i> Genetic Stock Center
SB3930	<i>hisB463 λ<sup>-</sup></i>	22
UTH780	<i>hisC780 malA1 (λ<sup>r</sup>) xyl-5 mtl-1 rpsL145 λ<sup>-</sup></i>	22, 24
WB353	<i>Δ(his-gnd) λ<sup>-</sup></i>	5, 33
SB3931	<i>hisF860 ara-14 galk2 malA1 xyl-5 mtl-1 rpsL145 λ<sup>r</sup> λ<sup>-</sup> supE44?</i>	24
JC411	<i>leuB6 fhuA2 lacY1 supE44 gal-6 hisG1 rfbD1? galP63? argG6 rpsL104 malT1 λ<sup>r</sup> xyl-7 mtl-2 metB1</i>	31
UTH903	<i>hisI903 malA1 λ<sup>r</sup> xyl-5 mtl-1 rpsL145 λ<sup>-</sup></i>	22, 24
<b>Plasmids</b>		
pIL253	Em <sup>r</sup> <i>L. lactis</i> high-copy-number vector	41
pBS	Amp <sup>r</sup> M13 ori pBR322 ori plasmid for sequencing	Stratagene
pHHH	<i>trpBA ΔhisH3 tyrA Cm<sup>r</sup></i>	D. J. Henner
pIL378	9-kb <i>Sau3A</i> segment of <i>L. lactis</i> DNA in pIL253	This work
pIL381	3.6-kb <i>Sau3A</i> segment of <i>L. lactis</i> DNA in pIL253	This work
pIL700	9-kb <i>SacI</i> segment from pIL378 in pBS	This work
pIL701	3-kb insert derived from pIL700 by spontaneous deletion	This work
pIL704	2.2-kb <i>EcoRI</i> segment from pIL378 in pBS	This work
pIL708	3.1-kb <i>EcoRI</i> segment from pIL378 in pBS	This work
pIL710	1.2-kb <i>EcoRI-XbaI</i> segment from pIL708 in pBS	This work
pIL712	1.9-kb <i>EcoRI-XbaI</i> segment from pIL708 in pBS	This work
pIL716	2.3-kb <i>HindII</i> PCR segment in pBS	This work
pIL717	1.6-kb <i>HindII-AsuII</i> segment from pIL700 in pBS	This work

DNA sequence analysis (reported below) indicated that the *his* genes might form an operon but that the 3' end of the putative operon was not present in pIL378. Inverse polymerase chain reaction was used to isolate this end. For this purpose, chromosomal DNA was cut with *HindIII* and ligated at a concentration of 10 μg/ml. For amplification, 0.2 to 1 μg of DNA was used with primers complementary to the sequenced region (see Materials and Methods). A 2.3-kb *HindIII* fragment was thus obtained and was cloned in pBS, yielding plasmid pIL716 (Table 1; Fig. 1).

**Complementation experiments.** The 9-kb insert carried by pIL378 was subcloned in pBS as indicated in Fig. 1. The resulting plasmids and pIL716 were used to complement various *B. subtilis* and *E. coli his* mutants. Seven genes involved in histidine biosynthesis were thus detected, organized in the following order: *hisC*, *-G*, *-D*, *-B*, *-A*, *-F*, and *-IE* (Fig. 1). However, the complementation pattern is not fully understood and probably depends on the presence of adequate promoters on the segment tested and/or appropriate interaction of the gene products with the host proteins. *L. lactis* subsp. *lactis* genes for branched-chain amino acid synthesis also gave inconsistent complementation in *B. subtilis* and *E. coli* (23).

**Nucleotide sequence of the *his* region.** The complete nucleotide sequence of a 11,160-bp region was determined (Fig. 2). Analysis of this sequence revealed the presence of 14 open reading frames (ORFs) (Fig. 1 and 2). The 5' ends of the

first and the last ORFs (ORF1 and ORF14, respectively) were not sequenced. All of the fully sequenced ORFs are preceded by a typical ribosome binding site, complementary to the 3' end of the *L. lactis* subsp. *lactis* 16S RNA (UUUCCUCC) (17). All ORFs except the last one (ORF14) are transcribed in the same direction. ORF1 is separated from ORF2 by 442 bp, whereas the following ORFs either overlap or are separated by less than 53 bp.

**Assignment of the ORFs.** The sequences of the proteins encoded by the ORFs were compared with those in the GenBank and EMBL data bases, using the FASTA and CITI2 softwares. Significant homologies (23 to 46.5% identity) have been found for 10 ORFs but not for ORF1, ORF6, ORF13, and ORF14. Eight ORFs encode proteins homologous to enzymes involved in histidine biosynthesis and were named according to the corresponding *E. coli* genes (Table 2). All proteins are of a size close to that of the *E. coli* counterparts except for the product of *hisB*, which has 46.5% identity with the carboxy-terminal region of the *E. coli* HisB protein. The product of ORF3 is homologous (23% identity) to the histidyl-tRNA synthetase (*hisS*) of *E. coli* but lacks about 150 carboxy-terminal amino acids (21). The product of ORF8 is homologous (28% identity) to the Apha-3' enzymes from various microorganisms and contains typical motifs present in these enzymes (26, 32, 43).

**Transcription signals.** A putative promoter (TTGACTN<sub>17</sub>TATAAT), showing homology with the consensus of the

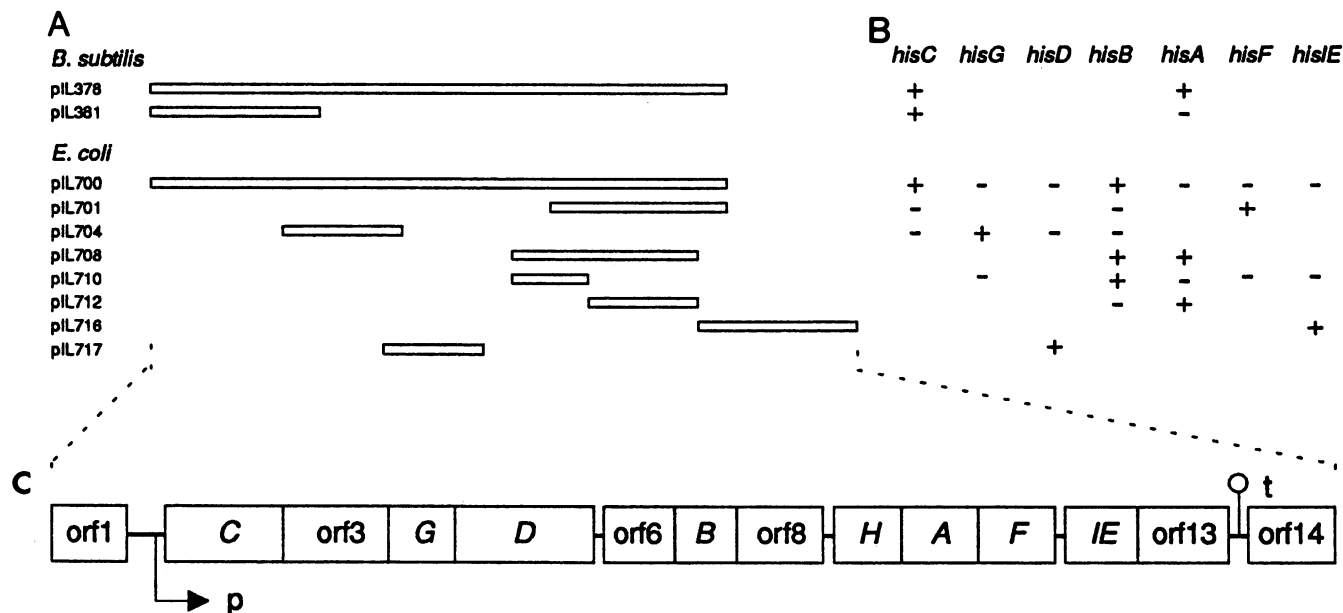


FIG. 1. Histidine region of *L. lactis* subsp. *lactis* NCDO2118. (A) Segments used for complementation. Numbers identify the plasmids that carry the segments. Plasmid constructs are described in Table 1. (B) Complementation experiments with *B. subtilis* and *E. coli* mutants listed in Table 1. Symbols indicate bacterial growth (+) or lack of growth (-) on a medium without histidine or supplemented with histidinol for *hisD* mutants. (C) Organization of the *his* region as deduced from sequence results.

lactococcal promoter (TTGACAN<sub>18</sub>TATAAT) (17), is present 340 nucleotides upstream of ORF2. A rho-independent transcription terminator-like structure is present between the two last ORFs (Fig. 2). No terminator structure was found between ORF1 and *hisC* in strain NCDO2118. However, the corresponding region of another strain of *L. lactis* subsp. *lactis*, IL1403, was also sequenced and found to be 97% homologous to that of NCDO2118. The nucleotide sequence of IL1403 contains a palindrome capable of forming a stem-and-loop structure upstream of *hisC* and the putative promoter (Fig. 2). This structure might possibly act as a terminator of transcription of ORF1.

## DISCUSSION

We isolated a cluster of genes involved in histidine biosynthesis in *L. lactis* subsp. *lactis* NCDO2118. Complementation studies in *B. subtilis* and *E. coli* indicated that at least seven *his* genes (*hisC*, -*G*, -*D*, -*B*, -*A*, -*F*, and -*IE*) are present within an 11-kb region. Sequence analysis of the region showed the existence of 14 ORFs, 8 of which are significantly homologous to *his* genes from various microorganisms. An additional *his* gene, *hisH*, was thus revealed. This finding indicates that all genes required for histidine biosynthesis except one, encoding histidinol phosphate phosphatase, are present in a single cluster in *L. lactis* subsp. *lactis*. In *E. coli*, the phosphatase activity is encoded by the *hisB* gene, which also codes for the imidazole-glycerol phosphate dehydratase. The resulting HisB protein is a bifunctional enzyme, with N-terminal phosphatase and C-terminal dehydratase domains (12). The *L. lactis* subsp. *lactis* *hisB* gene product is homologous to the C-terminal domain of the HisB protein only, and none of the other ORFs of the cluster codes for a product homologous with its N-terminal domain. This finding suggests that the phosphatase gene is located elsewhere in the *L. lactis* subsp. *lactis* chromosome. Alternatively, one of the ORFs encoding

a protein which is not homologous to the *E. coli* phosphatase might dephosphorylate histidinol phosphate (see below). Two different genes, mapping in different regions, code for the phosphatase and dehydratase in several other microorganisms, such as *S. cerevisiae*, *S. coelicolor*, and *Azospirillum brasilense* (42, 30, 20). For instance, *HIS2* and *HIS3* genes, which encode the phosphatase and dehydratase, respectively, map on chromosome R6 and chromosome R15 of *S. cerevisiae* (7). Genes encoding the dehydratase in *S. coelicolor* and *A. brasilense* have been sequenced and found not to code for the phosphatase (30, 20).

The eight *his* genes are part of a cluster of 12 ORFs that is preceded by a typical *L. lactis* subsp. *lactis* promoter consensus sequence (TTGACTN<sub>17</sub>TATAAT) and followed by a rho-independent transcription terminator-like structure. This finding suggests that the 12 ORFs may form an operon. ORF1, which is upstream of the putative promoter, is probably not a part of this operon, since it is rather distant (442 bp) from the next ORF, *hisC*, and is separated from the *hisC* promoter by a terminator-like structure in *L. lactis* subsp. *lactis* IL1403. This terminator is absent in strain NCDO2118 as a result of a 31-bp deletion which is a consequence of recombination between 6-bp direct repeats (Fig. 2). ORF14 is transcribed in the opposite direction from the other ORFs and is therefore not part of the operon.

Four ORFs of the postulated *L. lactis* subsp. *lactis* *his* operon encode products which have no apparent function in histidine biosynthesis. Two of them (ORF6 and ORF13) code for products which have no significant homology with the proteins present in the available data bases. In contrast, the product of ORF8 is homologous to the Apha-3' enzymes, which inactivate aminoglycoside antibiotics (43). Nevertheless, the presence of ORF8 under the control of P<sub>lac</sub> in *E. coli* failed to confer aminoglycoside resistance (kanamycin, tobramycin, butirosin, lividomycin, neomycin, dibekacin, amikacin, gentamicin, streptomycin, and spectinomycin were



R R K R I A S K Y P R V T K K Y F A Q K Q E D I E I I K L E G S V E L G P V V G  
AGACGTAAACGAATGCTTCAAATATCCAGAGTGACAAAAAATATTTGCTCAAAGCAAGAATATTGAAATTATCAAGTTGGAAGTTCGTGTGAGCTGGACCAGTTGTTGGT 3600

L A D A I V D I V E T G N T L S A N G L E V I E K I S D I S T R M I V N K S S F  
TTAGCTGATGCAATTTGACATTTGCGAACAGGAAATACCTTTATCTGCAAAATGGTTAGAGGTCATGAAAAATCAGTGACATTTCAACACGGATGATGTCAATAAATCTAGTTTC 3720

K F K K D K I I E M V E R L E D A Q T N \*  
AAATTTAAAAAGATAAAATTTATAGAAATGGTGGAGAGTTAGAAATGCTCAAACAAATGATTATCAAGGAAAGCTTGAAGAAATGCTGAAAAATTTCAAGTTCGAAAAACAGAAGT 3840  
RBS hisD ->

S K E V N K T V Q Q I V A D I Q K N G D T A L F N Y A K K F D G Y D V N T S N L  
ATCAAAAGAAAGTTAATAAACTGTTCAACAGATTGAGCAGACATTCAAAAAATGGAGATACTGCCTTATTTAACTATGCCAAAAAGTTCGACGGTTATGATGTGAATACTAGTAATTT 3960

L V T R M E R E A G L E Q I D E D Y F R I L R R T K S Q I E E F H K H Q L G N S  
ACTGGTCACGCCATGGAACGTGAAGCAGGACTAGAACAAATGATGAGGATTTTATTAGAATCTTAGACGCACCAATCACAATCGAAGAAATTCATAAGCACCACTGGGAAATTC 4080

W N I F K E N G V I M G Q I A R P L E R V A L Y V P G G T A A Y P S T V I M N A  
ATGGAATATTTTAAAGAAAATGGTGTATATGATGGGACAAATGCGCGTCTCTGGAACGTGTGCTCTCTATGTTCCCGGAGAACGGCTGCATATCCCTCAACAGTCAATATGAATGC 4100

V P A L L A G V K E I I M I T P V K A D G K V N P N I L A A A E V C G I E T I Y  
TGTCCAGCGCTTTAGCAGCGCTCAAGAAATTTATGATTAATCCAGTTAAAGCTGATGAAAAGTAAATCCAAATATTTAGCAGCTGCTGAAGTTGTGGAATAGAAAACATCTA 4320

K V G G A Q G V A A V A Y G T E S I P K V D K I V G P G N I F V A T A K K I C Y  
TAAGTGGTGGAGCACAAGCGTGTGCTGCGTTGCGTATGGGACAGAATCTATCCCAAAGTTGATAAGATTGTCGGACCCGGAATATTTTGTGGCTACAGCTAAGAAAATCTGTTA 4440

G V V D I D M I A G P S E V L V I A D K T A K P K Y I A A D L M A Q A E H D K L  
TGGGGTGGTAGATTTGATATGATACCGGTCGCTCAGAAGTTCTAGTTATGCTGACAAAACGCCAGCCAAAATATATCGCTGCTGATTTAATGGGCGAAGCAGAACATGATAACT 4560

A S A I L T V T T S E K L V Q Q V D E E L N R Q V Q N L E R R E I I E S S I R N Y  
TGGCTCAGCGCTTCTGACGACTTCTGAAAAACTTGTCAACAAGTAGAGAAATTAATAGACAAGTTCAAAATTTGGAACGTCGTGAAATTCATGAAATTCATGAAATTCATGAAATTC 4680

G G A I V V K N I D D A F D V S N Q L A P E H L E V L T S E P L T Q L P K I K N  
CGGTGGAGCCATTTGTTAAAAAATTTGATGATGCCCTTGTGTTTCCAATCAGCTGGCTCCAGAACATTTAGAAGTTTGTACTAGTGAACCTTTAAACCCAACTCCAAAAATCAAAAA 4800

A G S I F I G E Y T P E P L G D Y M S G S N H V L P T G G T A K F Y S G L G V Y  
TGCTGGCTCAATTTTATGAGAGATATACGCCAGAACCGTTAGCGGACTATATGTCAGGAAGCAATCATGCTTACCAACTGGAGGAAACAGCCAAATTTACTCTGGTTGGGTGTTTA 4920

N F I K Y L T Y S Y P K E V L A D F K E D V E T F A K S E G L T A H A N S I S  
TAATTTATAAAATATTGACTTATAGCTATTATCTCAAAGAAAGTTTGGCTGACTTAAAGAGGATGTTGAGACATTTGCAAAATCAGAAGGATTGACGGCTCATGCTAACTCAATTTTC 5040

V R F D E M \* M D F K I L N K K N S R E K N M  
TGTGAGATTGATGAAATGTAATACTTGTGAGACAAGATTTCAAATTTATGTTGTTCAAGGCGTGAAGGACCCACATGGATTTAAAAATTTGAATAAGAAAAATAGTAGGGAGAAAAACATG 5160  
RBS orf6 ->

T K Q E N Y Y A E V F E K P W G R M F Y D L L F P O L L P N L T K D S K I L S F  
ACAAAAAAGAAAATTTATACCGAGAAGTTTTCGAAAAACCATGGGTCGGATGTTCTATGACTTACTTTTCCACAGCTCTACCAAATTTGACAAAAGATTCAAATTTCTGAGTTTC 5280

G S G F G R T E T F L E E Q G F E V T G Y E P D V E K L E M M S D Q T F R O L T  
GGCTCTGGATTGGACGGACGGAACATTTTGGAGAACAGGATTTGAAGTACCGGCTATGAGCCTGATGTAGAAAAGCTCGAGATGATGTCTGACCAACATTTTCGTCAAGTGTACA 5400

G T F D D F A E T V K N E R Y D V I L I H N V L E Y V L D R K V V L E L L L S L  
GGAACTTTGACGACTTTGACAGAACTGTTAAAAATGAGCGTTACGACGTGATCTCATTACAATGTTTTAGAAATACGTCCTTGACCGAAAAGTCGTGTGGAATCTCTTGTCACTT 5520

L T D G G G T L S I V K H S K Y G S M I E A A A G R D N P Q A A L D V Y E N E A V  
TTGACATGGCGCACGCTTCTATTGTCAAKACAGTAGTACGGTAGCATGATAGAAATGGCAGCAGGACGTGATAATCCGACGACGCGCTTGTGTTTATGAAAATGAAGCTGTC 5640

A S H N H G D I L V Y D D D W L T D F V A N Y K L K L Q E K F G I R H F Y G I S  
GCTTCTCAACCCAGCGATATCTAGTTTATGACGATGATTGGCTGACAGATTTTGTGGCAAAATACAACTGAACTCCAGAAAAATTTGGAATTCGCTATTTTACGGTATTTC 5760

Q N A E I K E T E N W Y Q P M L K L E Q K V A K D O T L Y P V A R L H H L I F K  
CAAAACGAGAATCAAAGAACAGAGAACTGGTATCAACCCATGCTTAAGTTAGAGCAAAAAGTAGCGAAAAGACCAACGCTGATCCAGTCGCACGATTACATCATTGATATTTAAA 5880

K T K E N L L \*  
AAAATTAAGGAGATCTGTTATGACACGCATATCACACATCACGCGTAATACCAAGAAACACAAATCGAATTTCCATCAATTTGACGGCACAGGTCAAGCGGACATTAGTACAGGTA 6000  
RBS hisB ->

I G F L D H M L T L L T F H S D F D L K I I G H G D H E T V G M D P H H L I E D  
TTGGTTTTCTCAGCCACATGCTGACACTTCTCACCTTTCACAGCGATTTTGTACTAAAAATCATAGGACATGGGATCATGAAACAGTAGGGATGGACCCGCACCATCTCATTGAAGATG 6120

V A I A L G K C I S E D L G N K L G I R R Y G S F T I P M D E A L V T C D L D I  
TTGGATTGCTCTGGCAAATGATCAGCGAAGATTTAGTAATAAGCTCGTATTCGACGTTATGGAAGTTTACCAATCCAAATGGATGAAGCTTTTGTGACTTTGTGATTTAGATATTA 6240

S G R P Y L F H A D L S G N Q K L G G Y D T E M T E E F F R A L A F N A G I T  
TGGACGACCTTATTTGATTTCTAGTADTTTACAGGAAATCAAAAACTTGGTGGCTATGATACAGAAATGACTGAAGATTTTTCGTGCCCTTGTCTTTAATGCTGGGATACGT 6360

L H L N E H Y G O N T H H I I E G M F K S T A R A L K Q A V S I D E S K V G E I  
TACATCTGAACGAACATTTAGGGCAAAATACGCATCATATTTTGAAGGCATGTTTAAATCTACAGCAGAGCGCTAAAACAAGCTGTAAGTATTGATGAATCGAAAGTTGGAGAAATAC 6480

P S S K G V L \*  
CGAGCAGTAAAGGAGTGTATGACTAATCTAAAAGAAATTAAGAAATTAATATTGAGAAATTTCTGAAAGCATTCAACAATCGCTAAAAGATGCAAAAATATATGATAGCAGTTCTCTCC 6600  
RBS orf8 ->

E A Q V L F I D K K D G Y Y L K I A S S K T L E R E A E M T A Y F O K K K L G L  
TGAAGCTCAAGTGTATTGATGATAAAAAAGATGGCTATTATTTAAAAATAGCTTATCAAAAACCTTAGAGCGAGAAAGCTGAAATGACTGCTTATTTTCAAAGAAAAAGTTAGGTTT 6720

G Y I S Y L S D Q S Q D F L L K K K I O G E N Y L A K Q Y L N N P K R L C D N L  
AGGATATATTTCTTATTTATCAGACCGATCAGAGATTTTACTCAAGAAAAAATCAAGGAGAAATTTTGGCTAAACAATATCTTAATAATCCGAAACGCTGTGTGATAATCT 6840

FIG. 2—Continued.

A E N L R F L H E Q N F E D C P I L D H S E R Y L K K V E K N A S I N N S N L D TGCTGAAAATCTACGATTCTTCATGAACAAAATTTGAGGATTGTCCCATATTAGACCATTCTGAACGTTATCTGAAAAAGTCGAAAAGACGCAAGTATAAATAATCAAACTAGAA	6960
F V N N Y N I R T T E E A Y D Y I E N K K L L L R N D T L L H G D Y C L P N I I TTTTGTCAATAATATAATCCGAACAACAGAAGAAGCTTATGATTATATAGAAAAATAAAATGCTATTAAGGAATGATACGCTTTTACATGGAGATTATTGTTTACCAAAATAAT	7080
L D N W K F K G F I D L D C A G V G D R H I D L F W G A W T L N F N I G T D Q Y CCTAGACAATTTGAAGTTCAAAGTTTTATTGATTGGATTGTGCAGTGTGGCCAGCCACATATTGACCTCTTTGGGGTGCATGGACGCTTAATTTAATATTGGTACTGATCAATA	7200
R D R F F D A Y G R D R I D V D R L K L V G C C E V F G * TCGTGACCGATTTTTGATGCTTATGGTCCGGATAGAAATCGATGTTGATCGTTTGAATGGTGGCTGCTGTGAGGTTTTGGTTAAATGAATTTTAGTATATTTGATATTGAGG	7320
M K K I V I I D Y N I G N L Q S V Q A A F L R L G Q E T V I S R D L E E I R AGCATTATGAAAAATTTGTTATCATCGACTACAATATTGGAATCTTCAAAGTGTACAGCCGCTTTTACGATTGGGGCAGAAACAGTATTTCAGAGATTAGAGGAAATTCGT	7440
K A D A L I L P G V G A F P T A M N N L K K F N L I E L I Q E R A A A G A G I P I L AAAGCAGACTTATTCTCCAGGATGGGCTTTCCACAGCAATGAATAATTTAAAAAGTTAATTGTAACCTATACAAGAACGAGCTAGCTGGAATACCAATTTG	7560
G I C L G M Q V L F E K G Y E I E E R O G G L L K G E V I P I K T N E K I P H GGAAATTTGTTAGGATGCAAGTCTTTTGA AAAAGGATACGAGATAGAAAGAACAGGCTTGGACTTTTAAAGGGTGAAGTAATTCCAATCAAACTAATGAGAAAAATCCGCAT	7680
M G W N Q L N L A K T S P T T H Y L S G N D E V Y F V H S Y Q A T C P D D E L I ATGGGATGGAATCAATTAACCTGGCTAAAACAGTCCAACAACCTATTATTGTCTGGTAATGATGAGGCTATTATTGCTCCATCTTATCAGGCGACTGTCTGATGATGAATCATT	7800
A Y T T Y G E V K I P A I V G K N N V I G C Q F H P E K S G E I G R K I L K A F GCCTACACCATTATGGCGAAGTAAAAATCCGGCAATTTGGAAAAATAATGTGATAGGCTGTCAATTTCCACCTGAAAAGAGTGGAGAAATGGCAGAAAGATCAAAAAGCATT	7920
L E E I * M K I I P A I D L Q N G E A V R L Y K G D Y D K K T V Y S K N P L E I TTGGAGGAAATTTAAATGAAGATTATCCAGCAATGATTGCAAAATGGTGAAGCCGTGCTCTCAAAAGGAGATTATGATAAGAAAACGCTTATTCAAAAATCCCTTGAAAT	8040
A Q K F E R M G A T D L H L V D L D G A K I G Q T R N L E L V R K I K D E T R L GCTCAAAAATTTGAAAAGTGGGAGGACTGACCTCCATTGGTGTATTAGATGGTCTAAGATAGGACAACTCGTAATTTAGAGCTTGTGCGAAAAATAAAGATGAACAAGATTG	8160
K I E I G G G I R D F D T V R M Y L E Q I G V E R V I L G T A A V E K P D F L K AAAATCGAAATTTGGTGGAAATAGAGATTTCGATACAGTATAGATGTATCTTGAACAAATGGTGTGGAACGAGTATTAGGACCCGAGCGTAGAAAACCTGATTTCTTAAAG	8280
E L L I K Y G P S R I V V G V D I R E G F V S T S G W L E K T S L P Y L S F L K GAATTTAATAATTAATGTCGAAGCAGAATCGTTGTTGGAGTTGATATTAGAGAGGGTTTGTATCAACAAGTGGTGGTTAGAAAAACAAAGTCTTCCCTACCTGTCTTTTAAA	8400
K L E R I G V K T T I I T D I S K D G T L T G P N F K L Y D E I S K E N S L N V AAATTAGAGAAATAGGGTTAAAACACTATTATTACTGATATCTCAAAGACGGAACACTGCAGGTCCAAATTTTAACTTTATGATGAAATTTCAAAGGAAAATCCCTAAACGTG	8520
I I S G G V K D N S D I Q R A T R S D F Y G I I V G K A Y Y E G K I N L E K E F ATTATTCTGAGGTTAAAGGATAATCTGATATTCAACGTGCAACTCGTTCTGACTCTATGGAATATTCTGTTGGGAAAGCTTACTATGAGGAAAAATAATCTTGAAGGAGTTC	8640
M L T K R I I P C L D I K N G K V V K G I N F V G L R E I G D P V E L A K I Y R N A N * AGGAATGCTAACTAAAAGAATCATCCCTTGCTTGTATATAAAAATGGTAAAGTTGTTAAAGGAATCAATTTTGGGGTTTAAAGAAATAGGTGATCCAGTGAATGGCCAAAATATA	8760
E E Q C A D E I V F L D I T A S F E E R E I I G E L I G R A A R E L S I P L T V TGAAAGACAGTGTGAGATGAAATGTTTTCTTGATATTACAGCATCTTTTGAAGAAGCTGAAATTTTGGTGAATTAATGGTGGGGCGCGGTGAATTTCAATTCATTAAACGT	8880
G G G I R S I D D F R R I L A R G A D K V S V N S A A I E N P E L I R Q A A N E TGGTGAGGAATTCGTTCAATTGACGATTTTAAAGAAATCTTGTAGAGGAGCTGATAAAGTATCCGCTCACTCAGCTGCAATTTGAAAATCCCTGAACTATTCTGCAAGCGGTAACGA	9000
F G V Q C V V V A I D A K K R A D H R G Y D V Y I K G G R E N A G L D L V D W A ATTGGCGTTCAATGTTGTTGTTGCTATTGATGCTAAAAACGAGCAGACCATAGGGGATATGATGTCTATATCAAAGTGGGCTGAAAATGCAGGCTTGTACTTAGTTGATGGGC	9120
K K C E R L G A G E I L L T S M D K D G T K T G Y D L E M L N D V C T A V N I P TAAAAAGTGTGAAAGATTAGGAGCAGCGAAATTTACTAACCTCAATGATAGGATGGAACAAGACAGGCTACGATTTGGAATGCTGAATGACGTTGTACTGCTGTTAATATTC	9240
V V A S G G C G K I S D I V E V F Q N T R S D A A L F A S L F H Y G E E Q L M K TGTTGATGAAAGTGGCGTTGGCAAGATTTCCGACATTTGTAAGTTTTTCAAATACAAGAGCGATGCTGCCCTTTTGTCTCATTATTTCATTATGGCGAAGAACAGTTGATGAA	9360
L K T N L * GTTAAAGACGAACTATAAAAAATAATTTCCAGCAAGAATTATTAAGAGGAGACATTATGAGACGAGATTTTCATAAGCAGGAATTTTCTGTTATTGTTCAAGATTATCAAAACAA	9480
N Q V L M L A Y T N E V A F E K M L E T G E T W F W S R S R Q K L W H K G E E S ATCAAGTATAATGCTTGCCTATACAAATGAAGTGGCTTTTGA AAAATGCTTGAACCTGTTGAGACTGGTTTGGTCAAGTTCACGACAAAACCTTGGCACAAAAGGAGAAGATCAG	9600
G H F Q K I K G M R L D C D Q D T L L V F V E Q I G N A C H T G A Y S C F Y D E GTCATTTCAAAAATAAAGGAATGCGTTGGATTGCGACCAAGATACTTTACTGGTTTTTGTGAAACAAATGGTAATGCTGTGCATACCTGGTGTACTCTGTTTTATGATGAAC	9720
L I P F D D S D I F S E L E K Q I I D R K L H P V E K S Y T N Y L L G E G I D K TTATTCTTTGATGACTCTGATATTTTAGTGAGTTGGAGAAGCAATCATCGACCGAAAAGTTACATCCAGTCGAAAAATCTTATACGAATTTATTGCTGGAGAGGGCATTGATAAAG	9840
V L K K V G E E A S E V T I A S K N S D K G E L L G E I D D L L Y H L F V L M N TATTA AAAAAGTTGGTGAAGAAGCAAGTGAAGTACTATTGCTAGTAAAAATTCAGATAAAGGAGAACTTTTAGGAGAAATGATGATTACTTTATCATTTGTTGATTAATGAATC	9960
Q Q G I S L E E V R Q K A K E R H Q L E G N K K E F H T R T A D * M K K L D Y H AGCAAGGTTATCTTAGAAGAGTACGGCAAAAAGCAAAAGACGCGATCAGCTAGAAGGAAATAAGAAGGAATTTATCAAGGACGCGAGATTAATGAAAAATAGATTATCATT	10080
F H S H F S A D S E E L P R K H V I E A I A Y G L E E I C F T E H R D F Y F P G TCCACTCTCATTTTTCGGCTGATAGCGAGGAGCTCCAGAAAGCATGTGATAGAAGCTATGATATGTTTGAAGAAATTTGTTTTACAGACATCGAGATTTTATTTCCAGGAA	10200

FIG. 2—Continued.

M D F S L N L P E Y F Q E I N R L Q A E F K D E I N I K I G L E M G I D L R F K 10320  
 TGGATTTTCTACTAAATCTACCTGAGTATTTTCAAGAAATCAATCGATTACAAGCGGAATTTAAAGATGAAATTAATATAAAAAATCGGTTTGAAATGGGAATTGATTACGTTTAAAT  
 S E I N Q F I D S A P F D F V I A S V H E I G D I E V Y D G T E F Y L Q K T K E 10440  
 CAGAAATTAATCAGTTTATTGATTCGGCTCCTTTTGACTTTGTGATTGCCTCTGTCATGAAATGGAGATATTGAAGTTTATGACGGAACAGAAATTTATCTACAGAAAACAAAAGAA  
 E A Q R E Y L L A C L D V V Q N F E T Y N S F G H L D Y V A R Y G P Y T D K S I 10560  
 AAGCACAAGAGAATATCTTTAGCTTGTAGATGTTGTTCAAAATTTTGAGACTTATAATTCCTTTGGCCATTAGATTATGTTGCTAGATATGGGCCCTACACGGATAAATCAATAA  
 T F A E N R E I L F E I L R A L A S K G K A L E I N T R L F D D P K T G Q F Y S 10680  
 CGTTTGCAGAAATCGGGAGATTTTATTGAAATCTTGGCGGCTTAGCTTCAAAGGAAAAGCATTAGAAATAAACTAGATTGTTTGTATGATCCGAAAACGGCAATTTTATAGTG  
 D L L I N F K R L G G K F I T L G T D S H I A K R D W L S I H K A R T L I K K A 10800  
 ATTTATTGATTAATTTAAAAGATTAGGTGGAAAATTTATAACTTTAGGAACGGATAGTCDATATAGCAAAGCGTGATTGGCTTCAATTCATAAAGCAAGAACTTAAATAAAAAAGCTG  
 G F R E L A T F S G M K I D K N K K S I K E \* orf14 . . . . . 10920  
 GTTTTCGCGAATAGCACTTTTAGTGGGATGAAAATGATAAAAATAAAAGTCTATTAAGAATAATAGACTTTTTATATTATGATGCTAAATCTTCTCAAGTAGCGCAAGACGTTCT  
 . . . . . T2 < . . . . . \* S A L D K E L L A L R E  
 TTGAATCTTGTATTGATAGTTGGTGGGCTTTGATGTAACGGTTATTGGGATGAATCGACATTCATGTGAACAAGCACCAGATATTTGCTTCGTTTCTCTGAAGCTAACATTTG 11040  
 Q V E Q N S L Q H A K I Y R N N P H V R C E H S C A G L Y K A E N E E S A L M Q  
 TCTGTTACATCTGGATTTCCGCAATTGATGTAACGTTCCATCAACCAATCAGTCGCGACAATGACATGTTCTTTTGGTTGATTGGAAGCTGGGATTCGGCTATCAAA 11160  
 R N C E P N G C N I Y R E C P S G D F W D R G V I V H E K Q N I P V A I R S D F

FIG. 2—Continued.

tested) (data not shown). The function of this ORF is therefore not known. However, Apha-3' enzymes catalyze phosphorylation of a hydroxyl group, which is the opposite reaction to the dephosphorylation of a hydroxyl group and is carried out by the histidinol phosphatase activity of the *E. coli* HisB protein. Since the *L. lactis* subsp. *lactis* *his* operon contains no ORF homologous to the phosphatase-encoding region moiety of the *E. coli hisB* gene, it is possible that the product of ORF8 carries out this dephosphorylation, although kinases are usually not bidirectional enzymes.

One of the ORFs of the *L. lactis* subsp. *lactis* histidine operon (ORF3) shows a significant homology to the *E. coli hisS* gene, which encodes the histidyl-tRNA synthetase (21). This enzyme catalyzes aminoacylation of tRNA<sup>His</sup> molecules with histidine and is not involved in histidine biosynthesis (35). Its activity is necessary for translation, and its expression should therefore be independent of histidine biosynthesis. However, the ORF3 gene product is slightly shorter than the human, yeast, and *E. coli* histidyl-tRNA synthetases and contains no significant homology with the third motif present in all enzymes of class II tRNA synthetases, which is required for their activity (16, 34). This finding indicates that the ORF3 gene product has probably no histidyl-tRNA synthetase activity. Insertional inactiva-

tion of ORF3 does not affect cell viability (unpublished results), which shows that the product of this ORF is not essential. Recently, it has been shown that the tRNA synthetases can control their own synthesis by interacting directly with their transcripts (9, 38). Furthermore, because of the sequence similarity of the tRNA<sup>His</sup> and the *his* operon leader mRNA regions in *S. typhimurium*, it was suggested that histidyl-tRNA synthetase might contribute to controlling expression of the *his* operon (2). This hypothesis suggests that ORF3 could have a similar regulatory role in *L. lactis* subsp. *lactis*. Regulation by attenuation, observed for *E. coli* and *S. typhimurium his* operons, does not take place in *L. lactis* subsp. *lactis*, since the operon lacks both the required palindromic structure and a histidine-rich leader peptide (5, 28). These considerations encourage further studies of the role of ORF3 in the regulation of *his* gene expression in *L. lactis* subsp. *lactis*.

ACKNOWLEDGMENTS

We thank D. J. Henner and V. Sgaramella for a plasmid and a *B. subtilis* strain, respectively. We also thank C. Anagnostopoulos for critical reading of the manuscript and F. Haimet for artwork. This work was supported in part by BRIDGE contract Biot-CT91-0263 of the Commission of the European Communities.

TABLE 2. Conservation of proteins involved in histidine biosynthesis between *L. lactis* subsp. *lactis* and various organisms

Organism compared	% Identical amino acids <sup>a</sup>								Reference(s)
	HisA	HisB	HisC	HisD	HisF	HisG	HisH	HisIE	
<i>Escherichia coli</i>	36.4	46.5 <sup>b</sup>	29.1	38.8	44.4	29.8	39.2	40.5	10
<i>Klebsiella pneumoniae</i>						26.3 <sup>c</sup>			39
<i>Azospirillum brasilense</i>		46.9					35.5 <sup>d</sup>		20
<i>Bacillus subtilis</i> <sup>e</sup>			26.3						25
<i>Streptomyces coelicolor</i>	36.8	44.5	29.8	39.2			37.1		30
<i>Halobacterium volcanii</i>			25.9						14
<i>Methanococcus vannielii</i>	35.7							26.2 <sup>f</sup>	6, 15
<i>M. voltae</i>	37.5								15
<i>M. thermolithotrophicus</i>	38.1								44

<sup>a</sup> Calculated by Kanehisa software (45) as the ratio of perfect matches to the shorter protein length.  
<sup>b</sup> Alignment from position 164 of *hisB* of *E. coli* (dehydratase activity).  
<sup>c</sup> Alignment with the N-terminal partial sequence available in *K. pneumoniae* (100 amino acids).  
<sup>d</sup> Alignment with the N-terminal partial sequence available in *A. brasilense* (70 amino acids).  
<sup>e</sup> The *hisC* gene in *E. coli* and *hisH* gene in *B. subtilis* code for the same function (histidinol phosphate aminotransferase).  
<sup>f</sup> Partial alignment of 63 amino acids with the 136 amino acids of the HisI protein of *M. vannielii*.

## REFERENCES

- Altboum, Z., S. Gottlieb, G. A. Lebens, I. Polachek, and E. Segal. 1990. Isolation of the *Candida albicans* histidinol dehydrogenase (*his4*) gene and characterization of a histidine auxotroph. *J. Bacteriol.* 172:3898-3904.
- Ames, B. N., T. H. Tsang, M. Buck, and M. F. Christman. 1983. The leader mRNA of the histidine attenuator region resembles tRNA<sup>his</sup>: Possible general regulatory implications. *Proc. Natl. Acad. Sci. USA* 80:5240-5242.
- Bachmann, B. J. 1987. Linkage map of *Escherichia coli* K-12, p. 807-877. In F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umberger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol. 2. American Society for Microbiology, Washington, D.C.
- Bardowski, J., S. D. Ehrlich, and A. Chopin. 1992. Tryptophan biosynthesis genes in *Lactococcus lactis* subsp. *lactis*. *J. Bacteriol.* 174:6563-6570.
- Barnes, W. M., and E. Tuley. 1983. DNA sequence changes of mutations in the histidine operon control region that decrease attenuation. *J. Mol. Biol.* 165:443-459.
- Beckler, G. S., and J. N. Reeve. 1986. Conservation of primary structure in the *hisI* gene of the archaeobacterium, *Methanococcus vannielii*, the eubacterium *Escherichia coli*, and the eucaryote *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* 204:133-140.
- Broach, J. R. 1981. Genes of *Saccharomyces cerevisiae*, p. 653-727. In J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), *The molecular biology of the yeast Saccharomyces*. Life cycle in inheritance. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Bruni, C. B., M. S. Carlomagno, S. Formisano, and G. Paoletta. 1986. Primary and secondary structural homologies between the *HIS4* gene product of *Saccharomyces cerevisiae* and the *hisIE* and *hisD* gene products of *Escherichia coli* and *Salmonella typhimurium*. *Mol. Gen. Genet.* 203:389-396.
- Butler, J. S., M. Springer, J. Dondon, and M. Grunberg-Manago. 1986. Posttranscriptional autoregulation of *Escherichia coli* threonyl-tRNA synthetase expression in vivo. *J. Bacteriol.* 165:198-203.
- Carlomagno, M. S., L. Chiariotti, P. Alifano, A. G. Nappo, and C. B. Bruni. 1988. Structure and function of the *Salmonella typhimurium* and *Escherichia coli* K-12 histidine operon. *J. Mol. Biol.* 203:585-606.
- Chapman, L. F., and E. W. Nester. 1969. Gene-enzyme relationships in histidine biosynthesis in *Bacillus subtilis*. *J. Bacteriol.* 97:1444-1448.
- Chiariotti, L., A. G. Nappo, M. S. Carlomagno, and C. B. Bruni. 1986. Gene structure in the histidine operon of *Escherichia coli*. Identification and nucleotide sequence of the *hisB* gene. *Mol. Gen. Genet.* 202:42-47.
- Chopin, A., M. C. Chopin, A. Moillo-Batt, and P. Langella. 1984. Two plasmid-determined restriction and modification systems in *Streptococcus lactis*. *Plasmid* 11:260-263.
- Conover, R. K., and W. F. Doolittle. 1990. Characterization of a gene involved in histidine biosynthesis in *Halobacterium (Haloflex) volcanii*: isolation and rapid mapping by transformation of an auxotroph with cosmid DNA. *J. Bacteriol.* 172:3244-3249.
- Cue, D., G. S. Beckler, J. N. Reeve, and J. Konisky. 1985. Structure and sequence divergence of two archaeobacterial genes. *Proc. Natl. Acad. Sci. USA* 82:4207-4211.
- Cusack, S., C. Berthet-Colominas, M. Härtle, and R. Leberman. 1991. Sequence, structural and evolutionary relationships between class 2 aminoacyl-tRNA synthetases. *Nucleic Acids Res.* 19:3489-3498.
- De Vos, W. M. 1987. Gene cloning and expression in lactic streptococci. *FEMS Microbiol. Rev.* 46:281-295.
- Ephrati-Elizur, E., P. R. Srinivasan, and S. Zamenhof. 1961. Genetic analysis, by means of transformation, of histidine linkage groups in *Bacillus subtilis*. *Proc. Natl. Acad. Sci. USA* 47:56-63.
- Eriani, G., M. Delarue, O. Poch, J. Gangloff, and D. Moras. 1990. Partition of tRNA synthetases into two classes based on mutually exclusive sets of sequence motifs. *Nature (London)* 347:203-206.
- Fani, R., M. Bazzicalupo, G. Damiani, A. Bianchi, C. Schipani, V. Sgarrella, and M. Polsinelli. 1989. Cloning of histidine genes of *Azospirillum brasilense*: organisation of the *ABFH* gene cluster and nucleotide sequence of the *hisG* gene. *Mol. Gen. Genet.* 216:224-229.
- Freedman, R., B. Gibson, D. Donovans, K. Biemann, S. Eisenbeis, J. Parker, and P. Schimmel. 1985. Primary structure of histidine-tRNA synthetase and characterization of *hisS* transcripts. *J. Biol. Chem.* 260:10063-10068.
- Garrick-Silversmith, L., and P. E. Hartman. 1970. Histidine-requiring mutants of *Escherichia coli* K12. *Genetics* 66:231-244.
- Gibson, T. J. 1984. Ph.D. thesis. Cambridge University, Cambridge, England.
- Godon, J.-J., M.-C. Chopin, and S. D. Ehrlich. 1992. Branched-chain amino acid biosynthesis genes in *Lactococcus lactis* subsp. *lactis*. *J. Bacteriol.* 174:6580-6589.
- Goldschmidt, E. P., M. S. Cater, T. S. Matney, M. A. Butler, and A. Greene. 1970. Genetic analysis of the histidine operon in *Escherichia coli* K12. *Genetics* 66:219-229.
- Henner, D. J., L. Band, G. Flagg, and E. Chen. 1986. The organisation and nucleotide sequence of the *Bacillus subtilis* *hisH*, *tyrA* and *aroE* genes. *Gene* 49:147-152.
- Herbert, C. J., I. G. Giles, and M. Akhtar. 1983. The sequence of an antibiotic resistance gene from an antibiotic-producing bacterium. *FEBS Lett.* 160:67-71.
- Jasin, M., L. Regan, and P. Schimmel. 1983. Modular arrangement of functional domains along the sequence of an aminoacyl-tRNA synthetase. *Nature (London)* 306:441-447.
- Johnston, H. M., W. M. Barnes, F. G. Chumley, L. Bossi, and J. R. Roth. 1990. Model for regulation of the histidine operon of *Salmonella*. *Proc. Natl. Acad. Sci. USA* 77:508-512.
- Legerton, T. L., and C. Yanofsky. 1985. Cloning and characterization of the multifunctional *his-3* gene of *Neurospora crassa*. *Gene* 39:129-140.
- Limauro, D., A. Avitabile, C. Cappellano, A. M. Puglia, and C. B. Bruni. 1990. Cloning and characterization of the histidine biosynthetic gene cluster of *Streptomyces coelicolor* A3(2). *Gene* 90:31-41.
- Low, B. 1968. Formation of merioids in matings with a class of Rec<sup>-</sup> recipient strains of *Escherichia coli* K12. *Proc. Natl. Acad. Sci. USA* 60:160-167.
- Martin, P., E. Julien, and P. Courvalin. 1988. Nucleotide sequence of *Acinetobacter baumannii* *aphA-6* gene: evolutionary and functional implications of sequence homologies with nucleotide-binding proteins, kinases and other aminoglycoside-modifying enzymes. *Mol. Microbiol.* 2:615-625.
- Nagai, A., E. Ward, J. Beck, S. Tada, J. Chang, A. Scheidegger, and J. Ryals. 1991. Structural and functional conservation of histidinol dehydrogenase between plants and microbes. *Proc. Natl. Acad. Sci. USA* 88:4133-4137.
- Natsoulis, G., F. Hilger, and G. R. Fink. 1986. The *HTSI* gene encodes both the cytoplasmic and mitochondrial histidine tRNA synthetase of *S. cerevisiae*. *Cell* 46:235-243.
- Parker, J., and S. E. Fishman. 1979. Mapping *hisS*, the structural gene for histidyl-transfer ribonucleic acid synthetase, in *Escherichia coli*. *J. Bacteriol.* 138:264-267.
- Pattee, P. A., H. C. Lee, and J. P. Bannantine. 1990. Genetic and physical mapping of the chromosome of *Staphylococcus aureus*, p. 41-56. In R. P. Novick (ed.), *Molecular biology of the staphylococci*. VCH Publishers, New York.
- Piggot, P. J., and J. A. Hoch. 1985. Revised genetic linkage map of *Bacillus subtilis*. *Microbiol. Rev.* 49:158-179.
- Putney, S. D., and P. Schimmel. 1981. An aminoacyl tRNA synthetase binds to a specific DNA sequence and regulates its gene transcription. *Nature (London)* 291:632-635.
- Rodriguez, R. L., R. W. West, R. C. Tait, J. M. Jaynes, and K. T. Shanmugam. 1981. Isolation and characterization of the *hisG* and *hisD* genes of *Klebsiella pneumoniae*. *Gene* 16:317-320.
- Sanderson, K. E., and J. A. Hurley. 1987. Linkage map of *Salmonella typhimurium*, p. 877-919. In F. C. Neidhardt, J. L.



- Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol. 2. American Society for Microbiology, Washington, D.C.
41. Simon, D., and A. Chopin. 1988. Construction of a vector plasmid family and its use for molecular cloning in *Streptococcus lactis*. *Biochimie* **70**:559-566.
  42. Struhl, K. 1985. Nucleotide sequence and transcriptional mapping of the yeast *pet56-his3-ded1* gene region. *Nucleic Acids Res.* **13**:8587-8601.
  43. Trieu-Cuot, P., and P. Courvalin. 1983. Nucleotide sequence of the *Streptococcus faecalis* plasmid gene encoding the 3' 5-aminoglycoside phosphotransferase type III. *Gene* **23**:331-341.
  44. Weil, C. F., G. S. Beckler, and J. N. Reeve. 1987. Structure and organisation of the *hisA* gene of the thermophilic archaeobacterium *Methanococcus thermolithotrophicus*. *J. Bacteriol.* **169**:4857-4860.
  45. Wilbur, W. J., and D. Lipman. 1983. Rapid similarity searches of nucleic acid and protein data banks. *Proc. Natl. Acad. Sci. USA* **80**:726-730.
  46. Winkler, M. E. 1987. Biosynthesis of histidine, p. 399-411. In F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.