***Lactococcus lactis* glyceraldehyde-3-phosphate dehydrogenase gene, *gap*: further evidence for strongly biased codon usage in glycolytic pathway genes**

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The gene *gap*, encoding glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12), was isolated from a genomic library of *Lactococcus lactis* LM0230 DNA. Plasmids containing the *L. lactis* gene were able to complement a *gap* mutant of *Escherichia coli*. The nucleotide sequence of *gap* predicted a polypeptide chain of 337 amino acids for the enzyme and a subunit molecular mass of 36043. The codon usage in *gap* and four other glycolytic genes from *L. lactis* showed a high degree of bias, when compared with 84 other chromosomal genes. Northern blot analysis of total *L. lactis* RNA showed that *gap* hybridized strongly with a 1.3 kb transcript. The 5' end of the transcript was determined by primer extension analysis to be a C located 35 bp upstream from the *gap* start codon. These transcript analyses, and the orientation of the open reading frames in the DNA flanking *gap*, indicated that in *L. lactis* *gap* is expressed on a monocistronic transcript. Nucleotide sequencing indicated that the DNA adjacent to *gap* did not encode other glycolytic pathway enzymes. The DNA sequence flanking *gap* contained two open reading frames (ORF156 and ORF211) of unknown function. The 3' end of a *clpA* homologue was identified in the sequence upstream of ORF156. The location of *gap* on the *L. lactis* DL11 chromosome map was determined to be between map coordinates 0-530 and 0-660.

Keywords: *Lactococcus lactis*, glyceraldehyde-3-phosphate dehydrogenase, transcript analysis, Clp family, chromosome mapping

INTRODUCTION

Lactococcus lactis is a Gram-positive bacterium that is used extensively for making cheese. Lactose catabolism to lactic acid provides most of the energy needs of this bacterium. The production of lactic acid requires the interplay of three metabolic pathways: the phosphoenolpyruvate:phosphotransferase system, the tagatose-6-phosphate pathway, and the Embden–Meyerhoff–Parnas (glycolytic) pathway (Thompson, 1987). The glycolytic pathway is responsible for the net production of two ATP molecules per molecule of hexose consumed. There is

limited information regarding the structure, organization and expression of genes involved in the central metabolism of carbohydrates in *L. lactis*. Four of the *L. lactis* genes involved in glycolysis have been cloned and characterized. These are *pfk*, *pyk*, *ldh* and *tpi*, which encode phosphofructokinase (EC 2.7.1.11), pyruvate kinase (EC 2.7.1.40), lactate dehydrogenase (EC 1.1.1.27) and triosephosphate isomerase (EC 5.3.1.1), respectively (Llanos *et al.*, 1992, 1993; Griffin *et al.*, 1992; Cancilla *et al.*, 1995). Transcriptional analyses demonstrated that *pfk*, *pyk* and *ldh* are organized in an operon (designated *las*) and expressed as a tricistronic mRNA, whereas *tpi* was found to be monocistronic. These four glycolytic genes were found to exhibit a strong bias in their codon usage (Cancilla *et al.*, 1995).

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The GenBank accession number for the nucleotide sequence reported in this paper is L36907.

Glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) catalyses the reversible oxidative phosphoryl-

Table 1. Bacterial strains, plasmids and bacteriophage used in this study

Strain, plasmid or bacteriophage	Characteristics*	Source or reference
Strains		
<i>L. lactis</i>		
LM0230	Plasmid-cured derivative of <i>L. lactis</i> subsp. <i>cremoris</i> C2 (formerly described as <i>L. lactis</i> subsp. <i>lactis</i> C2)	Efstathiou & McKay (1977); Salama <i>et al.</i> (1991)
DL11	Prt ⁻ derivative of <i>L. lactis</i> subsp. <i>lactis</i> ATCC 11454	Tulloch <i>et al.</i> (1991)
<i>E. coli</i>		
JM107	<i>endA1 gyrA96 thi-1 hsdR17 supE44 relA1 Δ(lac-proAB)</i> (F' <i>traD36 proAB lacI^qZAM15</i>)	Yanisch-Perron <i>et al.</i> (1985)
SURE	<i>e14⁻(mcrA) Δ(mcrCB-hsdSMR-mrr)171 sbcC recB recJ umuC::Tn5(Km^r) mvrC supE44 lac gyrA96 relA1 thi-1 endA1[F' proAB lacI^qZAM15 Tn10(Tc^r)]</i>	Stratagene
DF221	<i>gap⁻</i> amber mutant derived from <i>E. coli</i> K-10	Hillman & Fraenkel (1975)
Plasmids		
pGEM3zf(+)	Ap ^r ; <i>ΔlacZ</i> , 3.2 kbp cloning vector	Promega
pJDC9	Em ^r ; <i>ΔlacZ</i> , 6.85 kbp cloning vector	Chen & Morrison (1988)
pMU5520	Ap ^r ; pGEM3zf(+) with 200 bp fragment of <i>gap</i> prepared by PCR amplification of <i>L. lactis</i> LM0230 DNA	This study
pMU5521	Em ^r ; pJDC9 with 1.9 kbp <i>EcoRI</i> fragment containing part of <i>L. lactis gap</i>	This study
pMU5522	Em ^r ; pJDC9 with 2.0 kbp <i>HindIII</i> fragment containing part of <i>L. lactis gap</i>	This study
pMU5523	Em ^r ; pJDC9 with 3.1 kbp <i>NruI</i> fragment containing <i>L. lactis gap</i>	This study
pMU5524	Em ^r ; pJDC9 with 3.1 kbp <i>NruI</i> fragment in reverse orientation to pMU5523	This study
Bacteriophage		
λ70	λGEM11 with a 13.5 kbp partial <i>Sau3AI</i> fragment containing <i>gap</i> from <i>L. lactis</i> LM0230 in the <i>XhoII</i> site	This study

* Km^r, Tc^r, Ap^r and Em^r indicate resistance conferred to kanamycin, tetracycline, ampicillin and erythromycin, respectively.

ation of glyceraldehyde 3-phosphate to produce 1,3-bisphosphoglycerate, using NAD⁺ as a coenzyme (Harris & Waters, 1976). This enzyme, in conjunction with 3-phosphoglycerate kinase (EC 2.7.2.3), forms a central link between ATP production and NAD⁺ reduction, and therefore has a key role in glycolysis. This has been underscored by metabolic studies which showed that the decrease in glycolytic activity observed after starved *L. lactis* cells were provided with an energy source was primarily due to diminished glyceraldehyde-3-phosphate dehydrogenase activity (Poolman *et al.*, 1987). Since the publication of the amino acid sequence of lobster glyceraldehyde-3-phosphate dehydrogenase (Davidson *et al.*, 1967), the primary structures and predicted sequences of glyceraldehyde-3-phosphate dehydrogenases from many organisms have been reported, and all of the sequences share considerable similarity (Fothergill-Gilmore & Michels, 1993). Antisera directed against the glyceralde-

hyde-3-phosphate dehydrogenases of *Streptococcus faecalis*, *Pediococcus damnosus* and *Lactobacillus acidophilus* were used to demonstrate the relationships between five genera of Gram-positive lactic acid bacteria (London & Chace, 1983).

Given the key role of glyceraldehyde-3-phosphate dehydrogenase in production of lactic acid by glycolysis, studies were performed to isolate and characterize *gap* from *L. lactis*. In this report we describe the construction of a molecular probe, the cloning and sequencing of *gap*, and its transcriptional organization. Codon usage in *gap* and other glycolytic genes from *L. lactis* is discussed with respect to codon preference in *L. lactis*.

METHODS

Bacterial strains, plasmids, bacteriophage, and culture conditions. The bacterial strains, plasmids and bacteriophage used

in this study are listed in Table 1. *L. lactis* and *Escherichia coli* were grown as described previously (Cancilla *et al.*, 1995) except that *E. coli* DF221 was maintained on Fraenkal II medium (Hillman & Fraenkal, 1975) and grown on 56/GLC (half-strength minimal medium 56 of Monod *et al.*, 1951, supplemented with 0.4%, w/v, glucose) in complementation experiments.

DNA and RNA techniques. Methods used for plasmid and bacteriophage DNA purification, isolation of *L. lactis* genomic DNA, oligonucleotide synthesis, nucleotide sequencing and analysis, restriction endonuclease digestions, Southern and Northern hybridizations, and primer extensions were as described previously (Cancilla *et al.*, 1995).

Amplification of a molecular probe for *gap*. Degenerate oligonucleotides of the following sequences were used as primers in the polymerase chain reaction (PCR): GAPDH-1, 5'-TG(TC)AC(TCAG)AC(TCAG)AA(TC)TG(TC)(TC)T-(TCAG)GC(TCAG)CC-3'; and GAPDH-2, 5'-AC(AT)GC-(TC)TT(AT)GC(AT)GC(AT)CC(ATC)GT-3'. GAPDH-1 and GAPDH-2 encode highly-conserved amino acid sequences found in most bacterial glyceraldehyde-3-phosphate dehydrogenases (Fothergill-Gilmore & Michels, 1993). GAPDH-1 encodes CTTNCLAP (approximately residues 151 to 158), and GAPDH-2 encodes TGA AKAV (approximately residues 209 to 215) on the antisense strand. The PCR mix (50 µl final volume) consisted of template DNA (25 ng), degenerate oligonucleotide primers (250 ng of each), deoxynucleoside triphosphates (200 µM each), 1× *Taq* DNA polymerase buffer (Boehringer Mannheim), and 1 U *Taq* DNA polymerase (Boehringer). The reaction was carried out in a thermal sequeencer (model FTS-1C, Corbett Research) as follows: 95 °C for 3 min, and then 35 cycles of 45 °C for 1 min, 72 °C for 1.5 min, and 90 °C for 0.5 min.

SDS-PAGE. SDS-PAGE of crude cell extracts was carried out using the Phast system (Pharmacia LKB) according to the procedures recommended by the manufacturer. The size of the glyceraldehyde-3-phosphate dehydrogenase subunit was determined by comparison of its electrophoretic mobility with those of a set of molecular mass standards of 14 to 94 kDa (Pharmacia LKB).

RESULTS

Molecular cloning of *gap*, the gene encoding glyceraldehyde-3-phosphate dehydrogenase

Alignment of the known amino acid sequences of glyceraldehyde-3-phosphate dehydrogenases from different organisms revealed a number of highly conserved regions (Fothergill-Gilmore & Michels, 1993). The sequences of two of these regions were used to design degenerate oligonucleotide primers for the production of a specific probe for *gap* by PCR. When PCR was carried out with these primers (designated GAPDH-1 and GAPDH-2; see Methods) and *L. lactis* LM0230 DNA template, a single PCR product of 200 bp, the size expected from the amino acid sequence alignments, was obtained. The 200 bp PCR product was cloned (pMU5520) and its nucleotide sequence was determined. The sequence contained an open reading frame (ORF) with a predicted amino acid sequence exhibiting between 45 and 63% identity with part of the sequences of glyceraldehyde-3-phosphate dehydrogenases from other bacteria. The 200 bp PCR

Fig. 1. (a) Restriction map of the *L. lactis* DNA in λ 70. (b) Restriction map of *gap* and flanking DNA. Only the relevant sites are shown for *Sau*3AI (Sa), *Hind*III (H), *Nru*I (N), *Eco*RI (E), *Ssp*I (Ss), *Dra*I (D), *Bst*II (B), *Pst*I (Ps), *Pvu*II (Pv) and *Eco*RV (Ev). The solid lines beneath the map in (b) indicate DNA fragments described in Table 1; the solid boxes indicate the positions of the DNA fragments used as hybridization probes. The relative positions (and orientations) of *gap*, and other ORFs, are indicated by arrows. The location of oligonucleotide *mrc8B* used in primer extension analysis is indicated.

product was used as a specific probe for *gap* to screen a λ GEM11 genomic library of *L. lactis* LM0230 (Llanos *et al.*, 1992). Seven out of the 780 clones in the library hybridized strongly with the probe. DNA was isolated from these clones, digested with *Eco*RI, and Southern-blot analyses were carried out on the digests to identify a clone containing the entire *gap* gene (data not shown). This clone, designated λ 70, was used to construct a restriction map for the *L. lactis* DNA surrounding *gap* (Fig. 1a).

Nucleotide sequence of *gap* and flanking DNA

Restriction fragments containing *gap* and its flanking DNA were subcloned from λ 70 into pJDC9, and their nucleotide sequences were determined. The 3244 bp nucleotide sequence obtained is shown in Fig. 2. The largest ORF in this sequence (354 codons, bp 1199 to 2260) contained the nucleotide sequence determined previously for the 200 bp *gap* probe. Using the first ATG in this ORF as the translation initiation codon, the deduced amino acid sequence shared between 45 and 68% identity with the sequences of glyceraldehyde-3-phosphate dehydrogenases from other bacteria (Fig. 3). Curiously, the regions used to design the degenerate oligonucleotides used in the PCR were not completely conserved in the glyceraldehyde-3-phosphate dehydrogenase of *L. lactis* (positions 156 and 212; Fig. 3). This sequence homology provided strong evidence that the 354 codon ORF was the gene *gap*, encoding glycer-

ORF137' > T K S V L G Q L G D F F S P E F M N R P D
 CGAAGGACGAACTAAATCAGTCTGGTCTACGACGAGGATTTCTTAGCCGTAATCATGAACGCTTTGGA 72
 G I I E F S A L S K E N L L K I V D L M L D E V 144
 CGGAATATTAATGAACTCTCTGCTTTAAGCAAGAAATCTCTTAAATTTGTTGATTTAATGCTTGATGAAGT
 N E Q I G R N D I H L S V T Q A A K E K L V D L 216
 CAATGAACAAATTTGGAGTAATGATATTCATCTTAGCGTCACTAAGCGCGTAAAGAAAAATAGTGGACTT
 G Y N P A M G A R P L R R I I Q E N I E D S I A 288
 AGGAATATACTCGCAATGGGTGCAAGACTCTCTGAGAAATTTCAAGAAAAATTTGAAGACTCAATTGC
 D F Y I E H P E Y K Q L V A D L I D D K I V I S 360
 TGATTCTACATTCGACATCTCTGAATATAACAATTTGGTAGCTGATTGATTGATGATAAAAATGTCATTTC
 N O T Q E T A E T T D E E V P A E * 432
 TAATCAAAACCAAGAAACAGCAAGAACTACTGACGAGAAAGTTCTGCTGAATAAAAAATTTTGTCACTAAA
 ATAGTATTAGTACAGAAACCAAGAAATAAGAAACAATGAAATTTTCAATCCATTTGCTCAATCACTCAT 504
 TTTTCTCATTCACAAATGCACTGCCGAAATCTGTGACGTAATTTTCAATCCATTTGCTCAATCACTCAT 576
 * D M E D I W E D
 CAAGTAGCGAGATTTGAATATATATGAGTTTCTGACGCACTTGGCGTACGATACATTTGACCAAGTTTC 648
 L Y G L N Y N Y Q T E D A V Q A L S V N S L T R
 TGACAAGAGTATTTTCAATCTCATCAAGCGCAGTTTGAATCGCAATGTTGTGCTTTACAGCACTCTGCAC 720
 V L T N E I D L A T T A C H Q A K G S E A G
 CAATAAAAATTTTGAAGAGTCTTGAATTTTGAAGAAATTTGTCACGGCCCTCAATGGCAGAAAGACATCAT 792
 I F N Q L L G Q S Q F I R G R G E I A L F V D Y
 AAAAACAATAATCATGAAGATCTTTATTTAGAGAGAGAGCCGCAATTTTTCAGTTGTTGAGCGTAAAAATG 864
 F S I D H L D K N L S F G G N K G T T S R L L G
 CTTCATCAAGAGGATTTGATTTATTTCTGAGATAAGAGGGTGAATTTGAGACGATTGACGAGTCAAG 936
 E D V L S K I I K K L Y S P S V K L R N A L A I
 TGATGACATCTGCTCGATTTTTCAGGAAGCTTGGCAAAATGATGAACGTAACAGATTTGTCACCAACG 1008
 S M T R N E P L R A L I L L V Y V S Q E W A S
 ATGATATTTTCATATAAAAACTCTCTTAACATTTTATGAAAAAATTGACACTAAATAAAAAAGTAGT 1080
 S L K M <ORF156
 ATCAATTTATTTAGAGATAAGAGATGTCTCCATTAACATAAATTCATTATTTATATATACCAAGCAGTT 1152
 GACCTGAAACAATGTTTCGTATTCGGAAGAGTGATATTTGTAGTAAAAAGAGAAATTTTCATCATCTAAA 1224
 gap: M V V K V G I N G F G R I G R L L A
 TAAAAAAGCGGAGTCACTCATCATGAGTGAAGTTGGTATTAAACGGTTTGGTGTATTTGGAGCTTTGGC 1296
 L R R I Q E V E G V E V A H I N D L T D P A M L 1368
 ATTACGTGCAATTCAGAAAGTTCAGAGGATTTGAATAGGCGACATCAATGATTGACAGACCCAGCAATGCT
 A H L L K Y D T T Q G R K G T V E V K E D G F 1440
 TGCACATTTACTTAAATATGATGACGATCAAGAGCGTTTAAAGGAACAGTTGAGGTCAAAGAGATGTTT
 D V N G K F V K V T A E R N P E D I Q W A D S G 1512
 TGATGTCAGCGAAAAATTTGTGAAGTCACTGATGATGATGATGATGATGATGATGATGATGATGATGATG
 V E I V L E A T G F F A T K E K A E K H L H P G 1584
 TGTGGAAATTTGTTCTGAAGCGACAGGTTCCTTTGCGATTAAGAAAAAGCTGAAAAACATTTGCTATCTG
 G A K K V L I T A P G G N D V K T V V F N T N H 1656
 TGGAGCTAAGAAAGTTTGTATGACAGCCTGGTGAATGATGATGATGATGATGATGATGATGATGATGATG
 T I L D G S E T V C T T N S L A P M A 1728
 CACAATTTCTGATGAAGTGAACAGTATTTCTGCGGTTCTGTTACTACAAATAGTTTAGCACCATTGGC
 D A L N K N F G V R G G T M T T V H S Y T G D Q 1800
 AGACCTATTAACAAAAATTTGGTGTGAAGGTGGGACATGACTACTGTCCATCATACATGAGTGAACCA
 M T L D G P H R G G D F R R A R A A E N I V P 1872
 AATGACATTTGATGCTGACATCGTGGTGGAGATTTCCCGCGTGGCGGTGCGAGCTGAAAAATATTTGCTC
 A S S G A A K A I G L V L P E L S G L M K G H A 1944
 AGCATCAAGTGAGCGGCTAAGCAATTTGGTCTTTGCTGAGCTTTCTGTTGATGAAGAGACATG
 Q R V S T P T G S T V L E V T V L E K H V T D 2016
 TCAACGTGTTTCACTCAACAGGATCAATTAAGTACTGTTGACAGTCTGAAAAACATGCTCACTGTTGA
 E I N E A M K K A A D E S F G Y N V D E I V S 2088
 TGAAATCAACGAAGCAATGAAGCTCTGCTGATGAAGTGGTGTGATGATGATGATGATGATGATGATGATG
 D I I G M A Y G S L F D A T L T E V T D L K D G 2160
 TGACATTTACGCGATGGCTTACGTTCTTCTTGAAGTGAAGTGAAGTGAAGTGAAGTGAAGTGAAGTGAAG
 G Q L V K T A A W Y D N E M S F T A Q L I R T L 2232
 TGGACAAATAGTAAAACTGCTGCTGTTGATGACATGAATGAGTTTCTGCTCAATTAATTCGTACCTCT
 E Y F A K I A K * 2304
 TGAATATTTTGCCAAAAATGCTAAATAAAAAATTTATGATTTATTTCTGAGAAATTTGTCAAGTGAAT
 GCTGACAAAAGCTTTGGGATATCGTAACTGCTAAGCGAAGTAAAGCTAATCAAGATTTAATATGTTGA 2376
 CACAGTAAAGCTTAAAGCTGTGATAAAAAATTTACTGACAGAGCTTGCCAGTAAATTTAATCTTTATTT 2448
 TATTTTAAATGACAAATTCATTAGTGAATTTGCTGATTTATTAACAATAATCATGCCATCTTTAATTTCCCA 2520
 * K V I I M G D K I E W
 AGGTCATTCATATGATGTTGTTGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT 2592
 P D N M N I H D Y F M V G N T H D I E H Q V C I
 GGCTGGAAGATCTTTAAGCGAAATTTTCTTTTTCGCTCTCTTGTGATGATTTCCAGTGTACAGCAGC 2664
 A P F D K L R I K K K E G E K N Y Y E V T V R A
 ATGACGAACGAGTACCTGGAATCTCACGCTGACAGAAAGACAACTTGGCACCTTCAACGGCCGATC 2736
 H R V V Y G P V E R D V S L C G E G G E V A A D
 TTGTACAGAAATGGTCAACCACTTTTGCATTTATCATGATTTTACGCAATTTTGAAGCTTTCTTAGTGGAA 2808
 Q V S H S V V K A N Y M I E R M K Y A E K P P I
 TTCTATCTCTCTCATGACTTCAGGTTCTATGAGATCAAAAGCGCAATGACTTTTGGAGCAAAACAG 2880
 E N G E E D V E P E N P I L V A I V K K L L G L
 TTGTGTAGCAGAGGCACTCCGCGCAAGAGTCCCATTTTTCAGCATTGACAGGCTCTTGAAGATTTGTG 2952
 Q N A A L G V G G R L G M K E A M V P D Q S N H
 CAAGATTTGACAAATTTTCCCTCAAGATGATGTTCTTCTGATGATGATGATGATGATGATGATGATGATG 3024
 L F Q L M K E G L I I D E D S L P L T V D N A V
 TTCACTAAGGTTGGGATCTCTTACAGAAATAATATGCTCATGCTAATCATGCTGCTTCTTAAATTAAT 3096
 E R L T P Y G E R I I I D D M S I M H S A K I L N
 TGCTTGAATTCATGCGGATTCATTAATAAAGTCTCTCTATCTTTTGAATATAATCAATTAATATAGC 3168
 A Q I E D R N M <ORF211
 ATATTTTAGCTGTGAAATGGTAAAAATGATTTAGAAGATTAATAATGAAATGATTTTATATCTATAGA 3240
 ATTC 3244

aldehyde-3-phosphate dehydrogenase. The nucleotide sequence predicted a chain length of 337 amino acid residues and a subunit molecular mass of 36043 Da for *L. lactis* glyceraldehyde-3-phosphate dehydrogenase. A putative ribosome-binding site (GGAGG) was identified 8 bp upstream from the *gap* initiation codon (Fig. 2).

The 5' flanking DNA of *gap* contained two converging ORFs (Fig. 1b) of 137 (Fig. 2; bp 1 to 415) and 161 codons (bp 551 to 1033) that were designated ORF137' and ORF156, respectively. ORF137' extended up to the end of the sequenced DNA and its predicted amino acid sequence shared 53% identity with the C-terminal residues of ClpA encoded by the plasmid-borne gene *clpL* from *L. lactis* (Huang *et al.*, 1993), 42% identity with the C-terminal residues of ClpA from *Bacillus subtilis* (Ogasawara *et al.*, 1994), and 34% identity with the C-terminal residues of the *E. coli* ClpA (Gottesman *et al.*, 1990a). These findings suggested that ORF137' was the 3' end of the chromosomal *L. lactis* *clpA* homologue. ORF156 had a likely ribosome-binding site (AAGGAG) 9 bp upstream from the first ATG codon in the ORF (Fig. 2). Using this ATG as the translation initiation codon, ORF156 was predicted to encode a 17.4 kDa protein which shared 33% identity with the putative Rrf2 regulatory protein of *Desulfovibrio vulgaris* (Rossi *et al.*, 1993) and 28% amino acid sequence identity with the putative 16 kDa protein of *E. coli* (Wolfe & Smith, 1988). As yet, the genetic and/or biochemical functions of these hypothetical proteins have not been established. The DNA between the translational stop codons of ORF137' and ORF156 contains an inverted repeat sequence (bp 472 to 510; Fig. 2). Transcripts of this region could form stem and loop structures with calculated free energy values (Tinoco *et al.*, 1973) of $-14.6 \text{ kcal mol}^{-1}$ (-61 kJ mol^{-1}), followed by UUUUUU. These features are indicative of a bi-directional rho-independent terminator (d'Aubenton Carafa *et al.*, 1990).

The flanking DNA on the 3' side of *gap* contains an ORF of 230 codons (bp 2485 to 3174; Fig. 2) oriented in the reverse direction to *gap* (Fig. 1b). This ORF (designated ORF211) has a putative ribosome-binding site (AAGGA) located 14 bp away from the first ATG codon (Fig. 2). Using this ATG as the translation initiation codon, ORF211 was predicted to encode a 23.8 kDa protein which shared 26% identity with the putative Fms protein of *E. coli* (Guillon *et al.*, 1992). The function of Fms has not been determined. The DNA between the stop codons

Fig. 2. Nucleotide sequence of *L. lactis* *gap*, and its flanking DNA. The deduced amino acid sequences are shown for ORF137' (above), ORF156 (below), *gap* (above) and ORF211 (below). The underlined C at bp 1212 indicates the start point of transcription for *gap* determined by primer extension analysis. The likely -35 and -10 hexanucleotides of promoters (van de Guchte *et al.*, 1992) are indicated. Double underlining indicates putative ribosome-binding sites which exhibit complementarity to the sequence 3'-UCUUUCCUCCA-5' at the 3' end of the *L. lactis* 16S rRNA (Ludwig *et al.*, 1985). Asterisks indicate stop codons. The arrows indicate an inverted repeat sequence that is a likely bi-directional rho-independent terminator (d'Aubenton Carafa *et al.*, 1990).

Zmo	MA	A	A A LSRPDSGL LVT	GSVEGN F F R SAH TYP	TTEGNDMVID	KIV	D	ANLPHKKL	D	M C
Eco	MTI		IVF AA KRSD..I IVA	L ADYM YM	S H D	DGHLI	KIR	D	ANLK DEV	DV A
Kpn			#IVF AA KRSD..I IVA	L AEYM YM	S H D	DGHLV	KIR	D	ANLK DEV	DV A
Sty			#IVF AA KRSD..I IVA	L AEYM YM	S H D	DGHLI	KIR	D	ANLK NEV	DV A
Bst	A		NVF AALKNDP..I VAV	NADG	SVH LDAE V NDGDVS	EII K		NLA GEI	D	V S
Bme	MA	I	NVF AALKNDP..I VAV	AN	SVH LDAE V DGSNLV	TIEIS	D	AQLS GKQ	V	S
Bsu	MA		NVF AALNPE..I VAV	AN	Q SVH LDAE S DGNLNV	TIE S	D	AKLS GKQ	V	S
Taq	M		QVF. LHSR .. L	NKT	SIYH P E AYDDQYLY D	AIRA VKD	KE P	EA	GV	I S
Cgl	MTIR		NFF AVL RN D.L VAV	NKT ST	F SIM LGQE YDD SIT G	RIA Y	D	KNLD AHN	D	I S
Tma	AR A		VY I Y RKNPDI VA	TKT	SVHKK P K YT NSLI D	EI F	PD	SKLP K L	DF	I S
Cpa	MT A		L P ..L VA	AK	F SS N EI	GA V	E	F AD	KLP	GEL IDV C
Spy	M		F NI .. TR	N	D	G E	N I S	D	N D	TD
Lla	MVVKVGINGFGRIGRLARRIQEVEG..VEVAHINDLTDPAHLKLYDTTQGRFKGTVEVKEDGFDVNGKFKVTAERNPEDIQWADSGVEIVLEATG									
con	M		INGFGRIGR	E	NDL	D	G	A	P	V E TG
Zmo	I	TNT	SA T.A	S AKG DRT	VYGVNHKDLTADDKIV	NA	C	VLHV QOKI	IVR L	F N RI QI. S..
Eco	L	L D T R	IT.A	VM G SKDNTPMF	KGA FDKYA..GQDIV	NA	C	L KVI D	IIE L	AT AT K V S K..
Kpn	I	L D T R	IT.A	VL G SKDNTPMF	RGA FDAYA..GQDIV	NA	C	L KVI D	IVE L	AT AT K V S K..
Sty	I	L D T R	IT.A	VL G SKDNTPMF	KGA FDKYA..GQDIV	NA	C	L KVI D	IIE L	AT AT K V S K..
Bst	R	TKR D A	E.A	I S AKVENI	MGV QDKY PKAHH	NA	C	F KV HQE	IVR M	NN RI L . K..
Bme		TKRAD A	E.A	I S ASDEDI I	MGV EDKY AANH	NAP	C	F KV DK	L R M	N QI L . K..
Bsu		TKRAD A	E.A	I S ANEEDI I	MGV EDKY AANH	NA	C	F KV DK	I R M	N QI L . K..
Taq	V	TDADK KA	E.	I AKGEDI I	MGV EAY PSRHHI	NA		VMKV EEA	EKAL	N RL L . K..
Cgl		TDANA KA	IE.A	I S AS EDA F	YGV ESY PENHN	GA	C	KV DK	IEN L	A RLH A . . .
Tma	V	RNR L	Q.A	I AKGEDI	IGC EDQ KP.EH I	CA	I	IVKV HEK	IVS ML	N RV L . K..
Cpa	TK	A	VRA.	V S A L I	V NED . T	GA	C	KV DK	IEK F	I A N N . K
Spy	K	A	AN	V	D . T	GA	C	K HDA	IQK L	I A N .
Lla	FFATKEKAEEKLHPGGAKKVLITAPGGNDVKITVFNINETILD.GSETVISAGSCTTNSLAPMADALNKNFGVKGGMITVBSYTGDQMTLDGP. ERGGD									
con	F	A	H	GAKKV	P	V	S	CTTN	AP	G TT H T Q D H D
Zmo	L	T	SASMI T T	R VA I	K KLD ISI	P DV LVDFTF	PQRTD AE	SVL	TGDMTGVLG	TDEPL R FYSDFHS T
Eco	W	GG G	SQ I S T	V K	N KLT M F	P NV VVD TVR	AA YEQ KA V		E.GEMKGVLG	TE DV T FN EVCT V
Kpn	W	GG G	SQ I S T	V K	N KLT M F	P NV VVD TVR	AASYE KK I		SE.GAMKGVLG	TE DV T FN EVCT V
Sty	W	GG G	SQ I S T	V K	N KLT M F	P NV VVD TVR	AA YEQ KA V		E.GEMKGVLG	TE DV T FN EVCT V
Bst	L	G	S I TTT	VA	K KLN M M	P NV VVD AE	E E V A L		E.GELKGILA	SEEP L RNYN STVS T
Bme	Y		I T T	VS	K KLN G M	P NV LVD AE D E	E V N L E		E.GDLKGILG	SEEP L G YN NINS T
Bsu	Y		I T T	VS	K KLN G M	P NV LVD AE NQE	AE V A L E		E.GDLKGILG	SEEP L G YN NKNS T
Taq	L		I I TTT	TA	S K RFD M L	P A	SDITAL KRE	AE V A L	E.GPLKGILA	TE LQ VMDFHS I
Cgl	L		V T T	VA	K KLD Y L	PVI	A D TFNTKSE	ES A I E	V.GE ETLA	SEEP L T VHDH I
Tma	L		V I TTT	VA	V VK KLD M I	P D	D TLVL ET	E V AV E	TE.GRLKGIIG	DEP TTFSGI
Cpa			VS I N T	AQ I	K KLD N	PV	V IS K N	E A E N.		TE A VV IS
Spy	L		G A N T	I	N KLD A	PV	V VT D N S	S	SN.D	 TE P V V S
Lla	FRRARAAENIVPASSGAAGAIGLVLPESGLMKGAQRVSTPTGSITELTVLEKHVTVEINEAMKAAD.ESFG....YNVDEIVSSDIIMAYGSL									
con	R	R	P	GAA A	V P	G	G	RV	S	K A Y V
Zmo	V	SRE A	LE.... K ARVV	WG SNRMVD	AAQM TL*	45.0%				
Eco		KAGIALND....NF	LVS	TGYSNKVLDLIAHIS	*	47.7%				
Kpn		KAGIALND....NF	LVS	#		48.4%				
Sty		KAGIALND....NF	LVS	#		48.4%				
Bst	I	LS M I	 KM VVS	TGYSHRVVDLAA	INAKGL*	49.2%				
Bme	I	LS M ME	 NM VIS	SGYSNRVVDLAQ	I AKGL*	50.3%				
Bsu	I	LS M ME	 SM VIS	SGYSNRVVDLAA	I KGL*	50.6%				
Taq	V	K KAL	 NM VF	WGYANRVADLV	LVLKGV*	50.9%				
Cgl	G	K K	S NT VVS	WGY C L LT	LV SQ LN*	51.5%				
Tma		I N I	 K V S	YGYSNRVVD	LLL M*	53.1%				
Cpa		KIV V.	S VS	Y S V	*	68.3%				
Spy		Q K MEV.	S VVS	Y V	*	76.8%				
Lla	FDATLIEVTDLKDGGQLVKTAAYDNEMSFTAQLIRTLEYFAKIAK*									
con	D			WYDNE						

Fig. 3. Homology among the known and deduced amino acid sequences of glyceraldehyde-3-phosphate dehydrogenases from *L. lactis* and other bacteria, and the Plr protein of *Streptococcus pyogenes*. The amino acid sequences are shown of the enzyme from: *Zymomonas mobilis* (Zmo) (Conway *et al.*, 1987), *E. coli* (Eco) (Branlant & Branlant, 1985), *Klebsiella pneumoniae* (Kpn) (Nelson *et al.*, 1991), *Salmonella typhimurium* (Sty) (Nelson *et al.*, 1991), *Bacillus stearothermophilus* (Bst) (Biesecker *et al.*, 1977), *B. megaterium* (Bme) (Schlöpfer & Zuber, 1992), *B. subtilis* (Bsu) (Viaene & Dhaese, 1989), *Thermus aquaticus* (Taq) (Hocking & Harris, 1980), *Corynebacterium glutamicum* (Cgl) (Eikmanns, 1992), *Thermatoga maritima* (Tma) (Schultes *et al.*, 1990), *Clostridium pasteurianum* (Cpa) (GenBank accession number X72219), *S. pyogenes* (Spy) (Lottenberg *et al.*, 1992), *L. lactis* (Lla) (this study), and the conserved amino acids from the above (con). The first amino acid for each protein is the NH₂-terminal amino acid. Spaces indicate positions where sequences are identical to that of *L. lactis*; dots indicate gaps inserted into the sequences to maximize the alignment; # indicates the extremities of partial sequences; double underlining indicates the conserved active-site cysteine from the crystal structure of the *B. stearothermophilus* enzyme (Biesecker *et al.*, 1977); asterisks show the COOH-terminal extremity of each sequence; the percentages show the amount of identity between each sequence and the *L. lactis* sequence.

of *gap* and ORF211 (bp 2260 and 2485) did not contain any inverted repeat sequences as observed between ORF137' and ORF156, suggesting that transcription of both *gap* and ORF211 is terminated by a rho-dependent mechanism (Platt, 1994).

The only other ORF larger than 100 codons within the sequenced DNA was one of 122 codons (bp 2611 to 2976). This ORF is totally embedded in ORF211, in the opposite orientation, and had no significant homology with any proteins in the GenPeptide database. We conclude from

this sequence analysis that no genes encoding other glycolytic pathway enzymes are adjacent to *gap* in *L. lactis*.

Expression of *L. lactis gap* in *E. coli*

To establish that we had indeed cloned and sequenced *gap*, we looked for the expression of glyceraldehyde-3-phosphate dehydrogenase in *E. coli* cells harbouring pMU5523 and pMU5524, using *E. coli* strain DF221, a *gap* amber mutant (Hillman & Fraenkel, 1975). *E. coli* DF221 cells are unable to grow if glucose is the sole carbon source, and as a consequence this strain has been used to clone *gap* from *E. coli*, *Bacillus stearothermophilus*, *Zymomonas mobilis* and *B. megaterium* (Branlant *et al.*, 1983; Conway *et al.*, 1987; Schlpfer & Zuber, 1992) by complementation.

Derivatives of *E. coli* DF221 containing pJDC9, pMU5521, pMU5522, pMU5523 or pMU5524 (Fig. 1b) were constructed by transformation and the ability of transformants to grow on 56/GLC medium was tested. Transformants containing portions of *gap* (pMU5521 and pMU5522) or pJDC9 (control) grew on Fraenkel II medium but not on 56/GLC. Transformants containing the entire gene (pMU5523 and pMU5524) grew on Fraenkel II medium and on 56/GLC, indicating complementation of the *gap* mutation of *E. coli* DF221. Crude cell extracts from transformants were examined by SDS-PAGE (data not shown). An increased intensity of staining in the region where a 36 kDa polypeptide would migrate was observed in extracts of *E. coli* DF221-(pMU5523) and *E. coli* DF221(pMU5524), compared with extracts of *E. coli* DF221 or *E. coli* DF221(pJDC9). These complementation and SDS-PAGE data together with the nucleotide sequence information established that the DNA of bp 1247 to 2260 in Fig. 2 corresponds to the *L. lactis* gene *gap*.

Transcriptional analyses

Northern analysis of *L. lactis* LM0230 RNA demonstrated that a *gap* probe hybridized strongly with a single 1.3 kb transcript (Fig. 4a). Primer extension from oligonucleotide mrc8B using total RNA as template yielded one cDNA product (Fig. 4b). The mobility of the product was identical to that of the product in the G track, corresponding to bp 1212 in Fig. 2. We concluded from this observation that the C at bp 1212 was the 5' end of the *gap* transcript. The opposite orientation of the ORFs flanking *gap* (Fig. 1b), the location of the 5' end of the *gap* transcript, and the experimentally determined size of 1.3 kb for the *gap* transcript (Fig. 4a), indicated that *L. lactis gap* is expressed as a monocistronic mRNA. Northern analyses (data not shown) of total *L. lactis* RNA using probes a, b and c (Fig. 1b) indicated that a 2.8 kb transcript hybridized with the ORF137' probe, a 0.7 kb transcript hybridized with the ORF156 probe, and transcripts of 0.8 kb (strong) and 1.5 kb (weak) hybridized with the ORF211 probe.

Codon usage in *L. lactis gap*

Codon usage in *L. lactis gap* exhibits a marked bias towards particular codons and away from others (Table

Fig. 4. Transcript analyses of *L. lactis gap*. (a) Northern blot analysis of *L. lactis* LM0230 RNA. Total RNA from *L. lactis* LM0230 was electrophoresed through a 1.2% (w/v) agarose/6% (v/v) formaldehyde gel and stained with ethidium bromide. RNA was transferred to a nylon membrane, and hybridized with 32 P-labelled 200 bp *gap* probe. The numbers on the left indicate the sizes of RNA standards that migrated to the positions indicated. The arrow indicates the 1.3 kb *gap* transcript. (b) Primer extension analysis of the 5' end of *gap* mRNA. The autoradiograph shows electrophoretic separation of: 10% of the primer extension reaction (lane 1), and 20% of the primer extension reaction (lane 2), of cDNA products generated as described previously (Cancilla *et al.*, 1995) using oligonucleotide mrc8B (5'-GTGCGCTACTTCAACTC-3') as primer. Lanes A, C, G and T, electrophoretic separation of the products of dideoxynucleotide sequencing using oligonucleotide mrc8B as primer and plasmid pMU5523 as a template. The number on the right corresponds to the nucleotide sequence numbering in Fig. 2.

2). Of the 61 amino acid codons, 12 are not used in *gap*. The low G + C content (37 mol%) of *L. lactis* DNA may explain the non-usage of codons with a G or C in the third position, but it cannot be the reason for the preference for T compared with A in the third position of Leu, Ile, Ala, Arg and Gly codons, or the preference for A compared with T in the third position of Pro codons.

Chromosomal localization and copy number of *gap*

*Eco*RI, *Dra*I, *Hae*III, *Nru*I, *Ssp*I, *Pvu*II, *Hinf*I, *Hind*III, *Sau*3AI, *Stu*I, *Hind*II and *Hpa*I digests of *L. lactis* LM0230 chromosomal DNA were resolved by electrophoresis through agarose, and transferred to a nylon membrane. The fragments on the membrane were hybridized with the 32 P-labelled 3.1 kbp *Nru*I fragment (Fig. 1b), and their sizes were determined (data not shown). In all cases, the measured sizes of fragments corresponded with the sizes predicted from restriction mapping (Fig. 1) and sequence data (Fig. 2). These observations indicated the presence of a single copy of *gap* in the *L. lactis* LM0230 chromosome. To locate the gene on the physical map of the *L. lactis* DL11 chromosome (Tulloch *et al.*, 1991), samples of high-

Fig. 5. Southern blot analysis of PFGE separations of restriction digests of high-molecular-mass *L. lactis* DL11 DNA. (a) DNA was digested in agarose with *Sma*I (lane 1), *Not*I (lane 2) and *Sgr*AI (lane 3) and then subjected to pulsed-field gel electrophoresis through 1.2% (w/v) agarose for 20 h at 200 V. The pulse time was increased linearly from 1 to 30 s. The gel was stained with ethidium bromide. The numbers on the left indicate the sizes (kbp) and migration positions of selected markers from λ DNA concatamers (Pharmacia LKB) and markers from a *Hind*III digest of λ DNA. (b) Southern blot of the gel in (a) after hybridization with 32 P-labelled 200 bp *gap* probe.

molecular-mass DNA from *L. lactis* DL11 were digested with *Sma*I, *Not*I and *Sgr*AI, separated by pulsed-field gel electrophoresis, and then transferred to a nylon membrane. The membrane was hybridized with a 32 P-labelled *gap* probe (Fig. 5). The probe hybridized with 130 kbp *Sma*I (lane 1), 750 kbp *Not*I (lane 2) and 130 kbp *Sgr*AI (lane 3) fragments. The 750 kbp *Not*I band corresponded to fragment NtA, while the 130 kbp *Sma*I band was SmD as described by Tulloch *et al.* (1991). As it has previously been shown that SmD is located within NtA, we concluded that the single copy of *gap* is located on the *L. lactis* DL11 chromosome within fragment SmD between coordinates 0.530 and 0.660 on the physical map (Tulloch *et al.*, 1991).

DISCUSSION

We have cloned and characterized *gap*, the gene encoding glyceraldehyde-3-phosphate dehydrogenase from *L. lactis* LM0230. A DNA probe constructed by the PCR was used to isolate a molecular clone containing *gap*, and to locate a single copy of *gap* on a physical map of the *L. lactis* chromosome. The identity of the gene was established by the complementation of an *E. coli gap* amber mutant with the *L. lactis gap*. The deduced amino acid sequence of *gap* shared significant homology with sequences of glyceraldehyde-3-phosphate dehydrogenase from other bac-

teria; the degree of identity ranged from 68% with the *Clostridium pasteurianum* enzyme, to 45% with the *Zymomonas mobilis* enzyme. Of particular interest was the observation that the *L. lactis* glyceraldehyde-3-phosphate dehydrogenase was most closely related (77% identity) to the streptococcal plasmin receptor (Plr). It was reported that Plr had glyceraldehyde-3-phosphate dehydrogenase activity, and it has been suggested that Plr is a member of the glyceraldehyde-3-phosphate dehydrogenase family of proteins (Lottenberg *et al.*, 1992).

In some eubacteria *gap* is clustered with genes encoding other enzymes of the glycolytic pathway. For example, in *Corynebacterium glutamicum* and *Bacillus megaterium* a gene cluster of *gap*, *pgk* and *tpi* has been discovered (Eikmanns, 1992; Schlpfer & Zuber 1992). Transcriptional analyses of the *gap-pgk-tpi* cluster from *C. glutamicum* identified transcripts corresponding to *gap* and *gap-pgk-tpi* (Schwinde *et al.*, 1993). In *Z. mobilis*, *gap* is in a bicistronic operon with *pgk*, and transcripts corresponding to *gap* and *gap-pgk* were characterized (Eddy *et al.*, 1991). In contrast, the active *gap* (*gapA*) of *E. coli* is not clustered with other genes encoding glycolytic pathway enzymes (Branlant & Branlant, 1985). Studies in *E. coli* have shown that *gapA*, *pgk* and *tpi* are located at 39, 63 and 89 min, respectively, on the genetic map (Bachmann, 1990), and that *pgk* is clustered with *fda* (encoding fructose, 1,6-bisphosphate aldolase, EC 4.1.2.13) and a second, cryptic *gap* (*gapB*) (Alefounder & Perham, 1989). The flanking DNA of *gap* from *L. lactis* did not encode other glycolytic pathway enzymes (Fig. 2). We have made a similar observation for the DNA adjacent to *tpi* in *L. lactis* (Cancilla *et al.*, 1995). Transcript analyses indicated that *L. lactis gap* is expressed as a 1.3 kb monocistronic mRNA. Previous studies demonstrated that *L. lactis tpi* is also monocistronic (Cancilla *et al.*, 1995). The two genes, *gap* and *tpi*, are located at least 1.148 Mbp apart on the physical map of the *L. lactis* DL11 chromosome (Tulloch *et al.*, 1991). These findings show that considerable diversity exists among bacteria with respect to the transcriptional organization of their glycolytic pathway genes.

ClpA is an ATP-dependent protease found in both prokaryotes and eukaryotes (Gottesman *et al.*, 1990b). The Clp family of proteins, ranging in length from 756 to 926 amino acids, are also assumed to function as molecular chaperones (Squires & Squires, 1992). Recently, *clpL* from a transposon-like structure on the lactose-protease plasmid of *L. lactis* CNRZ270 was characterized (Huang *et al.*, 1993). On the basis of sequence identity with the C-terminal residues of ClpL (Huang *et al.*, 1993), and other bacterial ClpA sequences (Ogasawara *et al.*, 1994; Gottesman *et al.*, 1990a), ORF137' is most likely the 3' end of the chromosomal *L. lactis clpA* homologue. Northern analysis revealed that this gene was expressed on a 2.8 kb transcript in *L. lactis* LM0230. Further genetic and biochemical studies are required to establish conclusively the identity of ORF137'.

Based on the different degrees of codon bias in 782 coding sequences, it was postulated that three classes (I, II and

Table 2. Codon usage in *L. lactis*

Amino acid	Codon	84 chromosomal genes†		<i>gap</i> , <i>tpi</i> and <i>las</i> operon‡		<i>lac</i> operon§	
		Sum	%	Sum	%	Sum	%
F	TTT	1053	74	32	48	52	50
F	TTC	363	26	34	52	53	50
L	TTA	878	30	9	7	24	13
L	TTG	646	22	45	36	56	31
L	CTT	812	28	63	50	73	41
L	CTC	241	8	9	7	10	6
L	CTA	195	7	0	0	14	8
L	CTG	145	5	0	0	1	1
I	ATT	1595	68	55	45	75	56
I	ATC	509	22	68	55	54	41
I	ATA	246	10	0	0	4	3
M	ATG	726	100	42	100	50	100
V	GTT	1043	50	97	63	81	46
V	GTC	364	18	15	10	29	16
V	GTA	424	21	35	23	50	28
V	GTG	235	11	6	4	16	9
S	TCT	528	26	24	26	27	35
S	TCC	95	5	0	0	1	1
S	TCA	668	33	52	56	46	59
S	TCG	102	5	1	1	4	5
P	CCT	382	37	18	34	27	36
P	CCC	80	8	0	0	1	1
P	CCA	507	49	33	62	40	53
P	CCG	75	7	2	4	8	11
T	ACT	645	38	64	60	40	36
T	ACA	665	39	40	37	52	46
T	ACG	188	11	2	2	11	10
A	GCT	964	41	113	58	88	46
A	GCC	338	14	12	6	21	11
A	GCA	826	35	61	31	69	36
A	GCG	208	9	10	5	13	7
Y	TAT	811	74	9	24	57	66
Y	TAC	287	26	29	76	30	34
*	TAA	60	72	5	100	6	86
*	TAG	8	10	0	0	1	14
H	CAT	393	73	13	41	24	52
H	CAC	148	27	19	59	22	48
Q	CAA	1068	86	40	100	42	84
Q	CAG	177	14	0	0	8	16
N	AAT	1242	77	32	40	55	58
N	AAC	369	23	48	60	40	42
K	AAA	1964	84	104	96	114	78
K	AAG	384	16	4	4	32	22
D	GAT	1347	75	59	59	92	71
D	GAC	461	25	41	41	37	29
E	GAA	1910	85	133	96	129	83
E	GAG	333	15	5	4	26	17
C	TGT	123	84	6	100	13	87
C	TGC	23	16	0	0	2	13
*	TGA	15	18	0	0	0	0
W	TGG	269	100	10	100	22	100
R	CGT	436	40	63	89	32	58
R	CGC	100	9	7	10	9	16
R	CGA	156	14	1	1	7	13
R	CGG	65	6	0	0	0	0
S	AGT	457	23	6	6	17	17
S	AGC	165	8	10	11	6	6
R	AGA	282	26	0	0	6	11
R	AGG	51	5	0	0	1	2
G	GGT	841	39	106	61	98	59
G	GGC	266	12	13	7	14	8
G	GGA	839	39	49	28	46	28
G	GGG	218	10	6	3	9	5
Total codons:		31 221		1761		2096	
Amino acids:		31 138		1756		2089	

* Stop codon.

† On the basis of the nucleotide sequences for 84 *L. lactis* chromosomal genes from the following GenBank accession numbers: M35374; X59250; M35865; M58315; M90760; M90761; M87483; L07920; L18760; M65867; M87840; M86245; U05215; M99413; L20851; L14679; M88106; X71132; M33770; D10168; M64860; Z29065; X74298; X75428; X76642; X62621; X71897; L27596; X69123; M87289; L12227; U04468; X52273; L27649; M65089; L11061 and ORF137, ORF156 and ORF211 (this study); ORF318, ORF365 and ORF507 (R. Llanos, Russell Grimwade School of Biochemistry, unpublished data).

‡ On the basis of the nucleotide sequences of *gap* (this study), *tpi* (Cancilla *et al.*, 1995) and the *las* operon (Llanos *et al.*, 1993).

§ On the basis of the nucleotide sequences of *lac* ABCDFEG (de Vos *et al.*, 1990; van Rooijen *et al.*, 1991).

III) of genes exist in *E. coli* (Médigue *et al.*, 1991). Nine of the eleven *E. coli* genes encoding glycolytic enzymes, including *pgi* (encoding phosphoglucose isomerase, EC 5.3.1.9), *pfkA* (encoding the major phosphofructokinase), *tpi* and *gapA*, belong to class II because they show a strong bias in their use of synonymous codons (Médigue *et al.*, 1991). Class II genes are expressed at high levels. Codon usage in *L. lactis gap* exhibits a marked bias towards particular codons and away from others. When these data are combined with codon usage data from *L. lactis tpi* and *pfk*, *pyk* and *ldh* (of the *las* operon), and compared with the codon usage in 84 lactococcal chromosomal genes, a strong bias in the codon usage of glycolytic genes in *L. lactis* is apparent (Table 2). The extent of this bias is much greater than that in any of the so-called eccentric genes noted in a previous compilation of 74 *L. lactis* genes, with the exception of the short, 16 codon gene *leuL* (Chopin, 1993). The plasmid-borne genes of the *lac* operon (involved in the utilization of lactose) did not exhibit the same degree of bias as that observed in the glycolytic genes (Table 2). In *E. coli* and *Saccharomyces cerevisiae*, highly biased codon usage is a property shared by strongly expressed genes (Gouy & Gautier, 1982; Sharp *et al.*, 1986). We therefore propose that *tpi*, *gap*, *pfk*, *pyk* and *ldh* are strongly expressed in *L. lactis*, in common with glycolytic genes in *E. coli*. The codon preferences in these *L. lactis* genes (Table 2) most likely reflect a bias in tRNA abundance (Kurland, 1991). Information regarding the optimal codon usage in *L. lactis* may be useful for identification of coding regions and prediction of gene expression (Gribskov *et al.*, 1984), and in the design of synthetic oligonucleotide primers for PCR amplification of *L. lactis* genes.

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