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Cloning and Expression of the *Lactococcus lactis purDEK* Genes, Required for Growth in Milk

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An operon containing the genes *purD* and *purE* and part of the *purK* gene was cloned from the facultative anaerobic gram-positive bacterium *Lactococcus lactis* by complementation of the *purD* mutation in *Escherichia coli* SØ609. The genes encode enzymes in the de novo pathway of purine nucleotides. The expression of the genes was regulated approximately 35-fold at the transcription level by the availability of purines in the growth medium. Deletion analysis of the nucleotide region upstream of *purD* indicated that a region of 145 bp is enough to give regulated expression of the reporter *lacLM* genes, which encode β-galactosidase. Deletion of a region 79 bp upstream of the transcription start point reduced the promoter activity 33-fold when incubated in a purine-free medium and to values below the detection limit when incubated in a purine-containing medium. No secondary transcription start points were mapped in or close to this region, indicating that a putative activator site and not a promoter was deleted or partly destroyed.

Lactic acid bacteria are used in the production of fermented foods. The facultative anaerobe bacterium *Lactococcus lactis* is the best studied of lactic acid bacteria but is also increasingly being used as a model organism representing gram-positive anaerobes. *L. lactis* is primarily used for the production of various cheeses and other fermented milk products, such as buttermilk. These fermentation processes are very complex and not easily controlled, and it is therefore of interest to modify *L. lactis* genetically in a controlled manner to improve and control lactate formation, bacteriophage resistance, flavor formation, etc. To obtain such modifications we want to increase our knowledge about regulated expression of genes in *L. lactis*. Several reports describe various regulated expressions triggered by external stimuli like change in pH (11, 27, 31), temperature (1, 6), osmotic pressure (14), Cl^- ion concentration (32), and sugar composition of the medium (25, 37).

In a previous study we showed that a purine-requiring mutant of *L. lactis* cannot grow in milk, whereas the wild-type strain can (5). Apparently there are not sufficient purine compounds in milk to support growth, and *L. lactis* therefore depends on its own de novo synthesis of purine nucleotides. The de novo synthesis of purine nucleotides requires in general 10 enzymatic steps leading to IMP, which functions as a precursor for both AMP and GMP nucleotides. The purine nucleotides can also be formed by salvage reactions from purine nucleosides and bases (39). Whereas the de novo pathway seems conserved among various organisms, the salvage pathways can vary more between organisms (23). The organization of genes involved in purine metabolism and the regulation of the expression of these genes have been primarily described for *Escherichia coli* and *Bacillus subtilis* (7, 30, 33, 36, 38, 39). Very little is presently known about purine metabolism in *L. lactis*. However, as mentioned above, purine-requiring mutants have been described previously as has the gene *hpt*, which encodes hypoxanthine guanine phosphoribosyltransferase, involved in the salvaging of purine bases (5, 21).

This work describes the isolation and characterization of a putative operon from *L. lactis* consisting of three genes, *purD*, *purE*, and *purK*, encoding enzymes in the de novo pathway of purine nucleotides. Also, we show that the expression of the operon is regulated, and it is suggested that the regulation is mediated at the transcription level by an activator.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth media. Table 1 shows the bacterial strains and plasmids used in this work. *L. lactis* was grown routinely at 30°C in M17 (35), the purine-free defined DN medium (5), or SA medium (12). *E. coli* was grown in Luria-Bertani medium or the phosphate-buffered AB salt medium (3) as previously described (21). When necessary, erythromycin was added to a final concentration of 1 mg/liter for *L. lactis* or 300 mg/liter for *E. coli* and ampicillin was added at 50 mg/liter for *E. coli*. As purine sources the following were added at the indicated final concentrations: adenine (15 mg/liter) hypoxanthine (15 mg/liter), and guanosine (30 mg/liter).

DNA isolation, manipulations, and sequencing. Plasmid isolation from *L. lactis* was done as described previously (24). For *E. coli* the Qiagen (Hilden, Germany) plasmid purification kit was used. Plasmid transformation of *L. lactis* and *E. coli* was performed as described previously (21). Isolation of bacteriophage DNA was done as previously described (34). Procedures for the use of restriction endonucleases, T4 DNA ligase, and calf intestine alkaline phosphatase were performed as recommended by the suppliers (Boehringer, Mannheim, Germany; Stratagene, La Jolla, Calif., and Promega, Madison, Wis.). The nucleotide sequence of both strands of DNA was determined with the Sequenase 2.0 sequencing kit (United States Biochemicals) together with the universal primers (Stratagene) or customized primers (Pharmacia). All nucleotide sequence data were processed, and the deduced amino acid sequences were compared by using the Genetics Computer Group software package (version 8.1) (4) and the SwissProt Protein Database (release 35.0).

Cloning of *purDEK*. The bacteriophage vector λZAPII *cI857*(Ts) (Stratagene), containing partial *Sau*3A-digested chromosomal DNA from *L. lactis* CHCC285, was provided by Egon Bech Hansen, Chr. Hansen A/S, Hørsholm, Denmark, as in vitro-packaged phage particles (21). The *E. coli* strain SØ609 *purD* (Table 1) was infected with phage particles containing the *L. lactis* DNA and plated on minimal medium agar plates containing glucose, Casamino Acids, and thiamine at 30°C. Two lysogens that grew without purines added (Pur^+) were isolated. From both these strains, phage lysates that could transduce SØ609 to Pur^+ at a high frequency were produced. The bacteriophage strain λLN3 was isolated from a single plaque of the transducing lysate. All bacteriophage handling was done as described previously (34). DNA from λLN3 was isolated; digested with *Kpn*I, giving fragments containing the insert in the λLN3-derived plasmid pBluescript SK(−); ligated; and transformed into SØ609. Ampicillin-resistant colonies that grew on purine-free agar plates were selected. These colonies were shown to

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TABLE 1. Strain and plasmid list

Strain or plasmid	Genotype and/or relevant feature(s)	Source or reference
L. lactis		
CHCC285	Wild type	21
MG1363	Plasmid free	9
MG1614	MG1363 Rif^r Str^r	9
E. coli		
DH5α	Φ80 *lacZΔM15* Δ(*lacZYA-argF*) *U169 recA1 endA1 hsdR17 supE44 thi-1 gyrA96 relA1*	10
LN3	SØ609/λZAPII-*purDEK*	This study
SØ609	*ara thi rpsL* Δ(*pro-gpt-lac*) *hpt deoD purD*	13
Plasmids		
pBluescript KS(+/−)	Cloning vector Ap^r	Stratagene
pIC19H	Cloning vector Ap^r	ATCC 37408[b]
pAK80	Ery^r; promoterless *lacLM*	11
pLN48	7.2-kb *Kpn*I fragment from LN3 inserted into pBluescript KS(−)	This study
pLN51	3.2-kb *Kpn*I-*Spe*I fragment from pLN48 inserted into pBluescript KS(+)	This study
pLN67	0.9-kb *Eco*RI fragment from pLN51 inserted into pBluescript KS(+)	This study
pLN71	0.9-kb *Hin*dIII fragment from pLN67 (*purD′*) fused to *lacLM* in pAK80	This study
pLN72	0.9-kb *Hin*dIII fragment from pLN67 (*orf1′*) fused to *lacLM* in pAK80	This study
p10[a]	Exonuclease III deletion derivative of pLN71	This study
pLN82	0.9-kb *Eco*RI fragment from pLN67 inserted in pIC19H	This study
pLN84 to pLN90	Exonuclease III deletion derivatives of pLN82	This study
pLN93 to pLN99	*Hin*dIII-*Bam*HI fragments from pLN84-90 fused to *lacLM* in pAK80	This study

[a] p19, p22, p27, and p30 are also exonuclease III deletion derivatives of pLN71 (this study).
[b] American Type Culture Collection, Rockville, Md.

have obtained the plasmid pLN48 [pBluescript SK(−) with a 3.2-kb insert] originating from λLN3. The insert was subsequently cloned as a *Kpn*I-*Spe*I fragment into the *Kpn*I-*Spe*I sites of pBluescript KS(+). The resulting plasmid, pLN51, could transform SØ609 to Pur^+.

Construction of plasmids containing various parts of the promoter region of *purDEK* fused to *lacLM*. An *Eco*RI fragment containing 846 bp of *purD* and upstream region including the promoter from the plasmid pLN51 was cloned into the *Eco*RI site of pBluescript KS(+), giving pLN67. The 846-bp fragment, including a part of the polylinker region, was removed from pLN67 by digestion with *Hin*dIII. The *Hin*dIII fragment was ligated to *Hin*dIII-digested promoter probe vector pAK80 containing the promoterless *lacLM* genes from *Leuconostoc mesenteroides*, which encode β-galactosidase (11). The resulting plasmid, pLN71, is shown in Fig. 1. To map the location of the promoter, a series of nested deletions of the 846-bp fragment was made with exonuclease III, which was included in the Erase-a-Base kit (Amersham). The plasmid pLN71 was digested with *Bam*HI. Following protection of the *Bam*HI overhangs from digestion with exonuclease III by using α-thio-deoxynucleoside triphosphates and Klenow enzyme, the plasmid was further digested with *Not*I, which cleaves within *purD* (Fig. 1). The plasmid was then digested with exonuclease III from the *Not*I site at 21°C for various times ranging from 1 to 10 min. All other steps recommended for the Erase-A-Base kit were performed as suggested by the supplier (Amersham). After ligation of the various deleted variants of the plasmid pLN71, the plasmids were transformed into *L. lactis* MG1363 and plated on DN medium agar plates containing X-Gal (5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid) for screening of β-galactosidase activity. Only a few plasmids were selected for further characterization (Fig. 1).

Another series of nested deletions of the 846-bp fragment was constructed from the opposite end. The *Eco*RI fragment of pLN67 was cloned into the *Eco*RI site of pIC19H in both orientations, giving pLN81 and pLN82 (Table 1). The plasmid pLN82 was cut with *Sac*I (3′ overhang) and *Cla*I (5′ overhang) and digested unidirectionally with exonuclease III from the *Cla*I site, at 30°C for 1 to 4 min. Following S1 nuclease digestion and ligation as recommended for the kit (Erase-A-Base; Amersham), the plasmid derivatives were transformed into *E. coli* DH5α selecting for ampicillin resistance. Plasmids were purified, and inserts were screened for size by double digestion with *Hin*dIII and *Bam*HI. Plasmids with inserts of 500 to 240 bp were further analyzed (pLN84 to pLN90). The actual deletion points in plasmids pLN84 to pLN90 were determined by nucleotide sequencing using the reverse primer (Stratagene). The inserts of pLN84 to pLN90 were cut out by digestion with *Hin*dIII and *Bam*HI and ligated into *Hin*dIII- and *Bam*HI-digested pAK80. Upon electroporation of the ligation mixes into MG1614 and selection for erythromycin resistance, plasmids were purified. The inserts in plasmids pLN93 through pLN99 (Fig. 1) correspond, one to one, to the inserts in plasmids pLN84 through pLN90.

Determination of β-galactosidase activity. The plasmid-containing strains were grown in 30 ml of DN medium supplemented with 1% glucose, erythromycin, guanosine, adenine, and hypoxanthine without shaking (purine excess). To obtain conditions of partial purine starvation, the bacterial culture was grown in the presence of excess purines as described above. At an optical density at 600 nm (OD_{600}) of 0.3 to 0.4, determined with a Spectronic Genesys 5 spectrophotometer, 1.5 ml of bacterial culture was centrifuged and washed twice in DN medium and resuspended in 1.5 ml of fresh medium without purines. Growth was continued for 1.5 h to an OD_{600} of ~1. A volume of 1 ml of bacterial culture was withdrawn at an OD_{600} of ~0.3 for the purine excess conditions and at an OD_{600} of ~1 for the purine-free conditions. Immediate addition of chloramphenicol to a concentration of 100 μg/ml stopped protein synthesis. The samples were put on ice until the β-galactosidase activity was determined and normalized to OD_{600}. The activity is given in Miller units (20).

RNA extraction. Cultures of CHCC285 were grown in 50 ml of SA medium containing 1% glucose with slow magnetic stirring. For purine excess conditions, the medium was supplemented with guanosine, adenine, and hypoxanthine. At an OD_{600} of ~0.4, 5 ml of culture was mixed with 5 ml of an EPS solution (60% ethanol, 2% phenol, and 0.9% NaCl) prechilled at −30°C. After centrifugation at 5,000 × *g* for 5 min at 4°C, the pellets were washed in EPS solution mixed 1:1 with 0.9% NaCl. The pellet was frozen at −80°C and lyophilized in a vacuum centrifuge without heating. The lyophilized pellet was ground with acid-washed glass beads (100-μm diameter; Sigma) on the tip of a melted Pasteur pipette. After dissolving the pellets in a buffer containing 10 mM sodium acetate and 300 mM sucrose at pH 4.8 (0°C), the solution was added to a solution of sodium dodecyl sulfate in 10 mM sodium acetate (pH 4.8), to a final sodium dodecyl sulfate concentration of 1%. The solution was equilibrated at 65°C and extracted three times with phenol-acetate, pH 4.8, at 65°C. The RNA was precipitated at −18°C after addition of sodium acetate to 300 mM and ethanol at 70%, centrifuged, dried, and redissolved in 25 μl of water.

Primer extension analysis. Approximately 5 pmol of oligonucleotide (5′-CC AAACGCTTTTTATATACACAGAC-3′) was radioactively labelled at the 5′ end with 10 pmol of [^{32}P]ATP (3,000 Ci/mmol) and T4 polynucleotide kinase. The reaction was performed in 50 mM Tris-HCl (pH 7.6), 10 mM $MgCl_2$, 5 mM dithiothreitol, 0.1 mM spermidine, 0.1 mM EDTA, and 10 U of T4 polynucleotide kinase in a total volume of 50 μl for 20 min at 37°C. The reaction was stopped by the addition of EDTA to 25 mM and heating at 70°C for 10 min. After mixing 10 μl of labelled primer with 10 μg of total RNA in a total volume of 20 μl, annealing was performed in the presence of 100 mM KCl, by heating to 90°C followed by a slow cooling to room temperature over approximately 30 min. Elongation was performed in 30 μl at 41°C for 30 min, with a SuperScript II reverse transcriptase (GibcoBRL) and the buffer conditions recommended by the manufacturer. The elongation products were precipitated with 3 volumes of ethanol, dried, and resuspended in 8 μl of the formamide loading buffer supplied with the ThermoSequenase DNA sequencing kit (Amersham). The sizes of the elongation products were determined by separation on a polyacrylamide DNA sequencing gel with a DNA sequencing ladder as a size marker followed by exposure to X-ray film.

Nucleotide sequence accession number. The nucleotide sequence of the *L. lactis purDEK* operon, shown in Fig. 2, is available from the EMBL nucleotide sequence database under accession no. AJ000883.

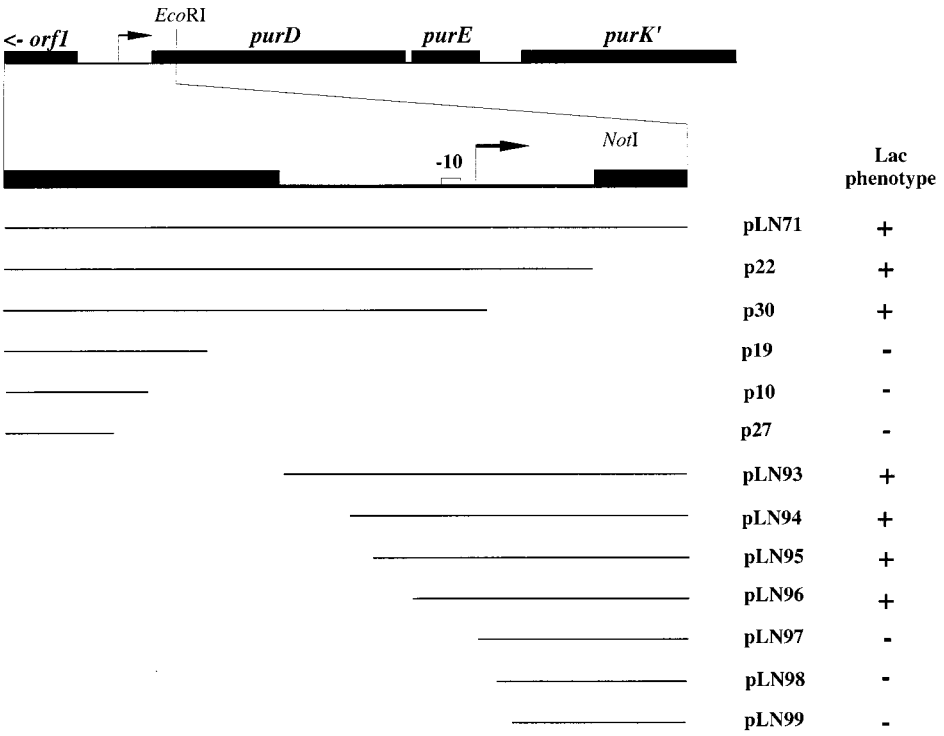

FIG. 1. Mapping the *purD* promoter by exonuclease III deletions. The structure of the *purDEK* region from *L. lactis* is shown on the top. Filled boxes correspond to the structural genes, and the open box corresponds to a putative −10 region. The localizations of DNA fragments present in the promoter probe plasmid pAK80 are shown below the physical map and are labelled by the plasmid designation. The Lac-positive (+) or -negative (−) phenotype (defined by the presence or absence, respectively, of blue color development on DN medium agar plates containing X-Gal) was analyzed in MG1363 transformants of the respective plasmid.

RESULTS

Cloning of *L. lactis purD* gene. A plasmid, pLN51, containing *L. lactis* chromosomal DNA was isolated as described in Materials and Methods by the ability to complement the *purD* mutation in *E. coli* SØ609. The nucleotide sequence of the insert of pLN51 is shown in Fig. 2. Four open reading frames can be found and are designated *orf1′* (nucleotide 364 to <1 [Fig. 2]), *purD* (nucleotide 720 to 1958 [Fig. 2]), *purE* (nucleotide 1991 to 2476 [Fig. 2]), and *purK′* (nucleotide 2527 to >3573 [Fig. 2]). Each of the reading frames is preceded by a good putative ribosome binding site (Fig. 2) except *orf1′* (18). Further downstream in the putative *orf1′* is located a GTG codon (nucleotide 250 [Fig. 2]) preceded by a better putative ribosome binding site (nucleotide 260 to 257 [Fig. 2]). Comparisons of the deduced amino acid sequences with amino acid sequences from the SwissProt Protein Database revealed high identity for the last three reading frames with the PurD, PurE, and PurK amino acid sequences from *B. subtilis*, *E. coli*, and *S. cerevisiae* (Table 2). A high degree of identity (30%) was found between the deduced amino acid sequence of the partially cloned *orfA′* and the amino acid sequence of the TetR protein from the *E. coli* transposon Tn*10*. However, this identity is not obvious if the start codon is located further downstream (nucleotide 250 [Fig. 2]).

Regulation of *purDEK* expression. The plasmid pAK80 contains a promoterless copy of the *lacLM* genes of *Leuconostoc mesenteroides*, which encode a β-galactosidase enzyme that is active in *L. lactis* (11). An 846-bp fragment (nucleotide 1 to 846 [Fig. 2]) containing the upstream region of *purD* was cloned in both orientations into plasmid pAK80 (Table 1) in front of the *lacLM* genes. The resulting plasmids pLN71 (*purD-lacLM*) (Fig. 1; Table 1), pLN72 (*orf1-lacLM*) (Table 1), and pAK80 were used for transformation of *L. lactis* MG1363. The strains MG1363/pLN71 and MG1363/pLN72 both gave blue colonies on X-Gal-containing agar plates, whereas MG1363/pAK80 gave white colonies. This suggested that there is promoter activity in each direction of the 846-bp fragment. The strains MG1363/pLN71, MG1363/pLN72, and MG1363/pAK80 were grown exponentially in the presence of adenine, hypoxanthine, and guanosine in the defined DN medium. After a shift to purine-free medium, β-galactosidase activity was measured (Table 3). The results showed that the expression of the β-galactosidase directed from the *purDEK* promoter in pLN71 was regulated approximately 35-fold depending on the available purines in the medium.

Mapping of the *purD* promoter by deletion analysis and primer extension. To locate the promoter with the purine-regulated activity on the 846-bp fragment in pLN71, a series of nested deletions of the insert were constructed as described in Materials and Methods. In Fig. 1 and Table 3 are shown the results of the analysis. The smallest plasmid with an upstream deletion that still has full regulation and activity is pLN95, whereas the smallest plasmid with a downstream deletion that has full regulation and activity is p30. This result indicates that only the region from nucleotide 450 to 594 (Fig. 2) is necessary for the regulation and activity and therefore contains the operator site and promoter. Deletion from nucleotide 450 to 497 (pLN95 and pLN96) (Table 3; Fig. 2) severely reduced expression, which indicates that either a binding site for an activator or the promoter is located within or near this region.

In order to map the transcription start point, we performed primer extension analysis on RNA extracted from the wild-type strain CHCC285 grown in the presence and the absence of purines (Fig. 3). A single band appears in the primer exten-

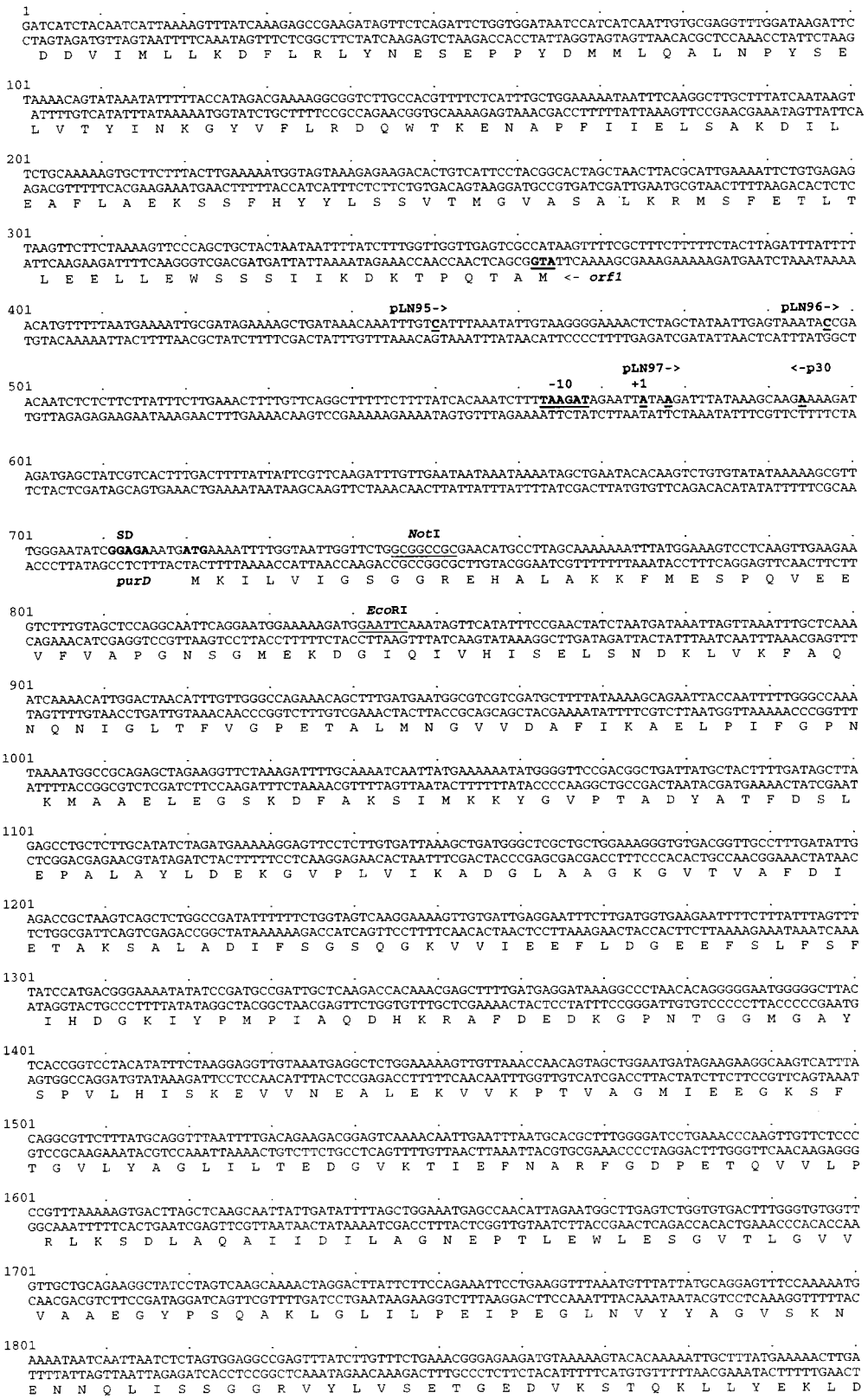

FIG. 2. Nucleotide sequence of the *purDEK* region from *L. lactis* CHCC285. The nucleotide sequence of the 3,573-bp *Sau*3AI fragment present in pLN51. The translated amino acid sequences of four open reading frames are shown below the nucleotide sequence and are labelled by the gene designation. Putative ribosomal binding sites (SD) are shown in boldface type. The transcription start point is underlined and marked +1, and the presence of a putative −10 region is likewise indicated by underlining. The locations of various endpoints from exonuclease deletions are indicated by underlining of the last nucleotide present in the deletion plasmids and an arrow above the nucleotide sequence pointing toward the other end of the DNA fragment in the particular plasmid (indicated beside the arrow).

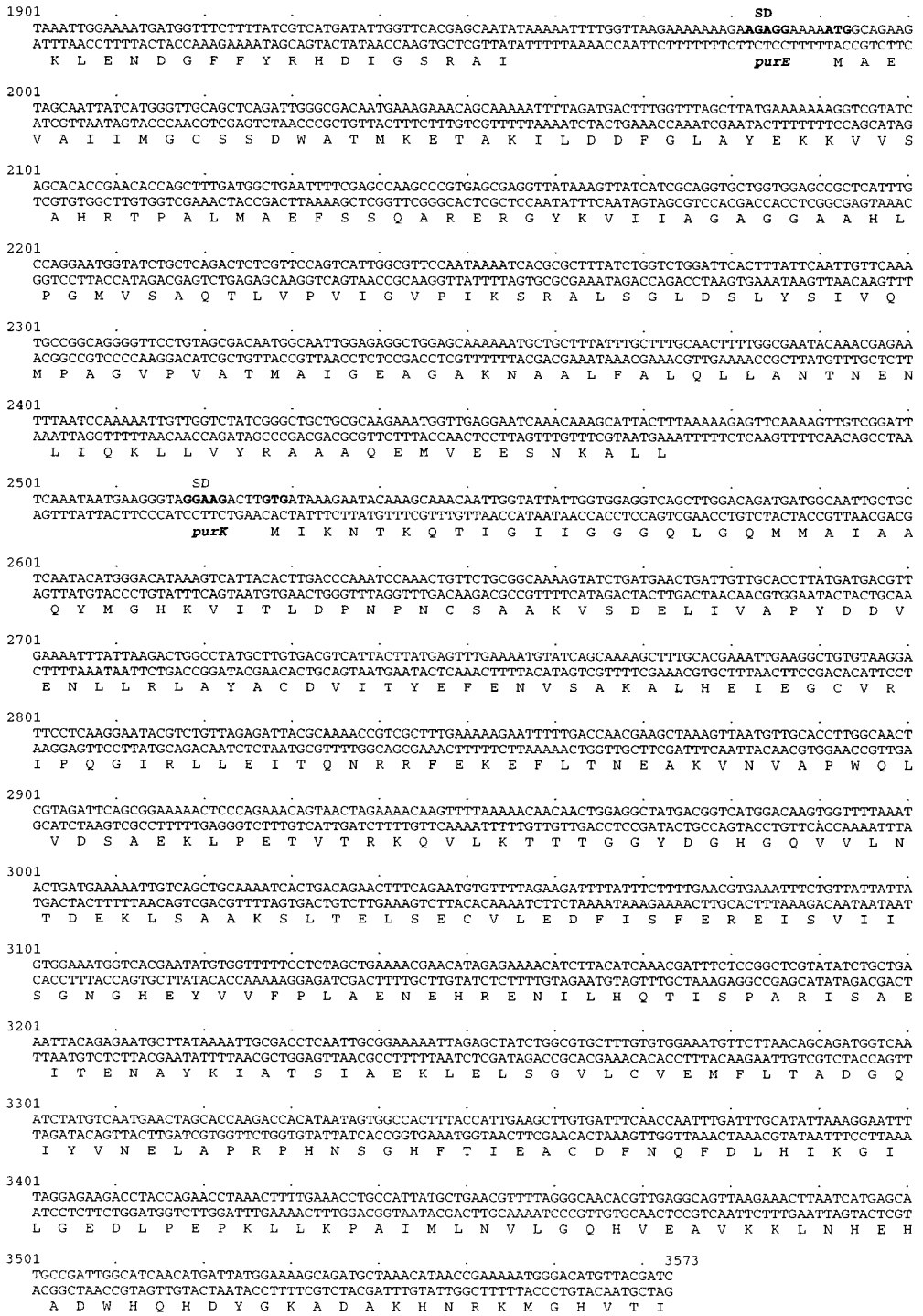

FIG. 2—*Continued.*

sion, at a position corresponding to a start point at the adenine nucleotide at position 575 (Fig. 2). This transcription start point is located far from the region between nucleotide 450 and 497, which is necessary for high expression (Table 3). However, the band shows up under conditions where the cells had been grown in the absence of purines (Fig. 3, lane 2), whereas in the presence of purines no band could be seen (Fig. 3, lane 1). These results are in accordance with the regulation of the specific β-galactosidase activities of MG1363/pLN71 under the same growth conditions (Table 3). Upstream of the mapped transcription start point (nucleotide 575 [Fig. 2]) a putative −10 region (TAAGAT, nucleotide 563-568 [Fig. 2]), −35 region (CTTTTTC, nucleotide 539-545 [Fig. 2]), and −44 region (TGTT, nucleotide 531-534 [Fig. 2]) are located. None of these regions are highly conserved in comparison with the consensus of some strong promoters (−44, AGTT; −35, CTT GACA; −15, TG; −10, TATAAT) found in *L. lactis* (22). Other sequences found between nucleotide 450 and the mapped

TABLE 2. Comparison of homologous Pur proteins

Origin of Pur homolog	% Identity to *L. lactis* protein[a]		
	PurD	PurE	PurK[b]
B. subtilis	54	61	45
E. coli	49	59	30
S. cerevisiae	39	52	34

[a] Percent identical amino acid residues after alignment of the deduced amino acid sequence of the indicated *L. lactis* Pur proteins with homologous sequences. Accession numbers for *B. subtilis*, *E. coli*, and *S. cerevisiae* sequences, respectively, are as follows: for PurD, P12039, P15640, and P07244; for PurE, P12044, P09028, and P21264; and for PurK, P12045, P09029, and P21264.

[b] The amino acid sequence deduced from *L. lactis purK'* is not complete.

transcription start point at nucleotide 575 show more resemblance to the consensus −35 and −10 regions; however, none of these are located near the transcription start point.

DISCUSSION

The *purDEK* operon has been cloned from the *L. lactis* subsp. *lactis* strain CHCC285, and its nucleotide sequence has been determined. Three reading frames showed extensive similarity to the *purD*, *purE*, and *purK* reading frames from other organisms. Unfortunately we did not obtain the last part of the *purK* gene, so we do not know whether *purK* is the last gene in the operon. A terminator structure is located after the *purK* gene in the *L. lactis* subsp. *cremoris* strain MG1363 (15), so we expect that *purK* might be the last gene in the *purDEK* operon in CHCC285.

The expression of the *purDEK* operon was regulated at the transcriptional level, giving approximately 35-fold higher expression in the absence of purines in the growth medium. This regulation of expression was shown by fusing the promoter to a reporter gene on a plasmid and would perhaps have been more pronounced if single copy expression were measured. However, it was possible to detect a transcript from the wild-type strain only when the strain was grown in the absence of purines and not in their presence, also showing the regulated expression in single copy.

Using two divergent sets of nested deletions we were able to localize the *purD* promoter and regulatory region between nucleotides 450 and 593. Deletions in this region (nucleotides 450 to 497) severely lowered the expression (Table 3). This indicated that a part of a promoter or a binding site for an activator is located within or near nucleotide 450 to 497 (Fig. 2). However, our primer extension analysis mapped the transcription start point to nucleotide 575 (Fig. 3), which makes it unlikely that the region contains the promoter. Thus, the region likely contains a binding site for an activator (26). A transcription activator was also found to be involved in the regulation of the expression of the *L. lactis purC* gene (17).

TABLE 3. β-Galactosidase production from fusion plasmids

Plasmid	β-Galactosidase activity (Miller units) from plasmid with:			
	Fusion point[a]		Addition to medium[b]	
	Upstream	Downstream	No Pur	Pur
pAK80			<1	<1
pLN71	1	846	390	13
pLN72	846	1	22	11
pLN95	450	846	270	6
pLN96	497	846	8	<1
pLN97	588	846	<1	<1
p30	1	594	530	13

[a] Fusion points correspond to the nucleotide numbers shown in Fig. 2.

[b] The basal growth medium was DN medium containing erythromycin (No Pur) or further supplemented with guanosine, adenine, and hypoxanthine (Pur).

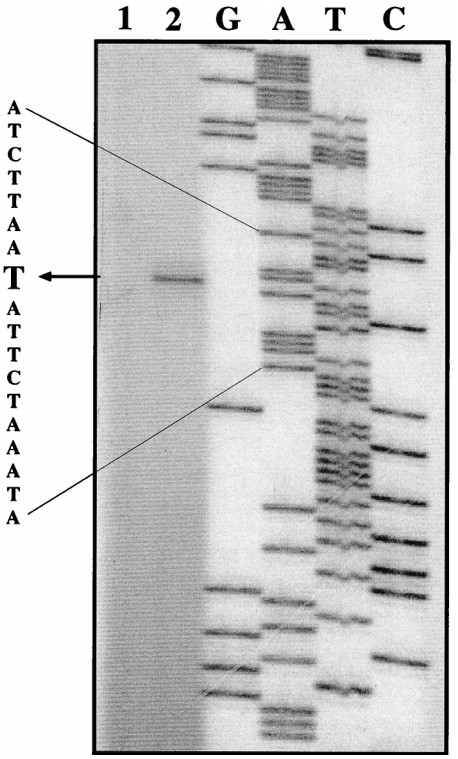

FIG. 3. Primer extension analysis of *purC* and *purD* transcription start points. Primer extension experiments were performed with 10 μg of RNA extracted from CHCC285 with primer MKP57 (5′-CCAAACGCTTTTTATATACACAG A-3′). RNA was extracted from cells growing exponentially in SA medium (lane 2) or in the same medium supplemented by purines (lane 1). Lanes G, A, T, and C contain sequencing reaction mixtures using the same primer as in the primer extension experiments and PCR-generated template DNA. The nucleotide sequence of the region around the transcription start point is shown, with the starting nucleotide in boldface type. The picture was scanned at 300 dots per in. by using a Scan Jet 4c/T device (Hewlett-Packard) and the DeskScan II version 2.3 software. The TIF file was imported into Top Draw (version 3.1) for the addition of text.

If the *purDEK* promoter is controlled by a transcription activator as it seems, it is a unique feature compared to other examined bacteria. In *E. coli* the regulation of purine de novo genes is by a repressor, PurR (16, 28, 29), which in the presence of the corepressors guanine or hypoxanthine (2, 19) binds to Pur box sequences in the promoter regions. The *B. subtilis pur* operon is regulated by two independent mechanisms, an attenuation mechanism utilizing an RNA binding protein (7) and repression of transcription initiation by a repressor (8, 36). The effector molecule for the attenuation control appears to be ATP (7), while 5-phosphoritosyl-α-1-pyrophosphate is an inducer of the PurR repressor (36). *L. lactis* has apparently evolved yet another mechanism for regulating its purine de novo genes in response to the purine availability.

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