Molecular Cloning of *Streptococcus bovis* Lactose Catabolic Genes

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The hydrolysis of lactose in *Streptococcus bovis* was shown to be catalysed by β -D-galactosidase. To isolate lactose catabolic genes, a gene library of *S. bovis* DNA was constructed in bacteriophage λ 47.1 and recombinants expressing β -D-galactosidase activity were detected using 5-bromo-4-chloro-indoyl- β -D-galactoside (X-Gal). The gene was cloned on a 7.8 kb DNA restriction fragment into pBR322 to generate the recombinant plasmid pHG1, which also encoded *S. bovis* lactose permease, thiogalactoside transacetylase and lactose repressor protein. The position and orientation of the four genes were determined by subcloning and transposon mutagenesis. The β -D-galactosidase, lactose permease and thiogalactoside transacetylase genes constitute an operon controlled by the repressor protein. β -D-Galactosides induced expression of the *S. bovis lac* genes in *Escherichia coli* while glucose had no effect. The nucleotide sequence of the presumptive regulatory region of the *S. bovis lac* operon was determined and compared with the corresponding *E. coli* sequence. The operators and the 5' end of the *lacZ* genes showed strong identity. The catabolite activator protein binding sequence, present in the *E. coli* promoter, was absent from the corresponding *S. bovis* region.

INTRODUCTION

It has recently been suggested that the genetic manipulation of certain rumen bacteria could result in an enhanced efficiency of livestock production (Smith & Hespell, 1983; Armstrong & Gilbert, 1985). Progress in this field is hampered by the limited genetic analysis of important rumen bacteria; consequently, we have initiated studies on the regulation of gene expression in the prominent rumen micro-organism *Streptococcus bovis*. Of particular interest is the characterization of genes involved in lactose catabolism. In most enterobacteria there are three *lac* genes organized in an operon. The closely linked *lacI* gene encodes a protein which represses *lac* gene expression. Some β -D-galactosides inactivate the repressor protein and so induce synthesis of the Lac proteins (Miller, 1978). Glucose represses expression of the *lac* operon by reducing cAMP concentrations. This metabolite activates the catabolic activator protein (CAP) which binds to the *lacZp* region and stimulates transcription of the operon. In *Staphylococcus aureus*, *Lactobacillus casei* and several streptococcal strains lactose is metabolized by a different pathway (Hengstenberg *et al.*, 1969). The sugar is phosphorylated during transport across the bacterial cell membrane. Cleavage by phospho- β -D-galactosidase yields glucose and galactose 6-phosphate. The latter is converted to dihydroxyacetone phosphate by three enzyme-catalysed reactions (Maeda & Gasson, 1986). Enzymes involved in this lactose catabolic pathway are plasmid-encoded in several Gram-positive bacteria (Lee *et al.*, 1982). The regulation of expression of these lactose metabolism genes is complex. In *Staphylococcus aureus*, galactose 6-phosphate is an inducer while in *Streptococcus lactis* expression appears to be regulated more by a repression due to the growth substrate. In *L. casei* a β -galactoside is required for induction. In

Abbreviations: IPTG, isopropyl thio- β -galactoside; X-Gal, 5-bromo-4-chloro-indoyl- β -D-galactoside.

all these organisms glucose represses the expression of the lactose genes (Lee *et al.*, 1982). The lactose catabolic pathway in *S. bovis* is unknown.

The aim of this study was to isolate and characterize the lactose catabolic genes from *S. bovis*.

METHODS

Microbial strains and plasmids. These are listed in Table 1. Bacteriophage were grown in *Escherichia coli* strains Q359, LE392 or MC1061, as described by Maniatis *et al.* (1982).

Media. *E. coli* strains were cultured in L-broth (1% tryptone, 0.5% yeast extract, 0.5% NaCl). *S. bovis* was grown in medium described by Silley & Armstrong (1984). Media were solidified with the addition of either 2% (w/v) Bacto-agar (Difco) or 0.8% agarose (Pharmacia). Glucose or glycerol, when required, were added to media at a final concentration of 2 mg ml⁻¹. Ampicillin (100 µg ml⁻¹) was used in media for the selection and growth of transformants. Functional β-D-galactosidase was detected by the addition of 5-bromo-4-chloro-indoyl-β-D-galactoside (X-Gal) to a final concentration of 40 µg ml⁻¹.

Chemicals. [α-³²P]ATP and the *in vitro* packaging kit were from Amersham. Restriction enzymes, DNA polymerase, DNAase and T4 DNA ligase were from BRL. X-OMAT RP X-ray film was from Kodak. Pharmacia supplied agarose. BA85 nitrocellulose filters were obtained from Schleicher and Schuell. All other reagents were from Sigma or BDH.

DNA isolation. (i) *S. bovis* DNA. Cells of *S. bovis* (10 g wet wt) grown to early stationary phase were harvested, washed three times with ice-cold 2 M-NaCl, resuspended in 20 ml 50 mM-Tris/HCl buffer, pH 8.0, containing 25% sucrose (w/v), 25 mM-EDTA (pH 8.0), 1 mg lysozyme ml⁻¹ and 50 µg mutanolysin ml⁻¹, and incubated for 15 min

Table 1. *Microbial strains and plasmids used in this work*

Bacteria, bacteriophage or plasmid	Genotype or phenotype	Source or reference
<i>E. coli</i> K12		
W3110	Prototrophic	This laboratory
CA159	<i>lacI</i>	This laboratory
LE392	<i>hsdR514 supE44 supF58 lacY1 galK2 galT22 metB1 trpR55</i>	This laboratory
MC1061	<i>hsdR rpsL araD139 Δ(ara-leu)7697 Δlac 74 galU</i>	Minton (1984)
JM101	<i>Δ(lac-pro) thi rpsL supE hsdR F' traD36 proAB lac^q ΔM15</i>	Viera & Messing (1982)
Q359	<i>hdsR_K supF (P2⁺)</i>	Gilbert <i>et al.</i> (1985)
NH4110	Harbours F plasmid carrying Tn1000 sequence	Guyer (1978)
<i>S. bovis</i>		
H/3		Silley & Armstrong (1984)
<i>Bacteriophage</i>		
λ47.1		Loenen & Brammar (1980)
M13mp8		Viera & Messing (1982)
M13mp9		Viera & Messing (1982)
<i>Plasmids</i>		
pACYC184	Tet ⁺ Cat ⁺	Chang & Cohen (1978)
pBR322	Bla ⁺ Tet ⁺	Bolivar <i>et al.</i> (1977)
pUC19	Bla ⁺ <i>lacZ'</i>	Norlander <i>et al.</i> (1983)
pNM52	<i>E. coli lacI^s Tet⁺</i>	Gilbert <i>et al.</i> (1986)
pNM480, pNM481, pNM482	Bla ⁺ <i>lacZ'</i>	Minton (1984)
pHG1, pHG2	Bla ⁺ . pBR322 containing <i>S. bovis lac</i> genes	This work
pHG3, pHG4, pHG5	Bla ⁺ . Derivatives of pHG1 with deletions of part of <i>S. bovis</i> DNA	
pHG6, pHG7	Bla ⁺ . Fragments of pHG1 cloned into pUC19	
pHG8	Cat ⁺ . Derivative of pACYC184 containing <i>S. bovis lacI</i> gene	
pHG9	Bla ⁺ . pHG1::Tn1000 with insertion at position 1.5	
pHG10	Bla ⁺ . pNM480 containing <i>S. bovis lacZpo</i>	

at 37 °C. After lysis of the cells by the addition of SDS to a final concentration of 1%, DNA was extracted as described by Gilbert *et al.* (1985).

(ii) Plasmid DNA. *E. coli* plasmids were purified from 1 litre L-broth cultures as described by Clewell & Helinski (1969). The rapid boiling method of Holmes & Quigley (1981) was used for small scale isolation for screening purposes.

Construction of a phage library of S. bovis DNA and screening of the library for the streptococcal β -D-galactosidase gene. *S. bovis* DNA was partially digested with *Sau*3A. DNA fragments 9–20 kb in size were purified by agarose gel electrophoresis and ligated to λ 47.1 DNA cleaved with *Bam*HI. The DNA was *in-vitro*-packaged and the *Spi*⁻ phenotype of recombinant phage was selected by growth on the *E. coli* P2 lysogen Q359 (Loenen & Brammar, 1980).

For screening the library, recombinant phages were grown on the *lac*⁻ *E. coli* strain MC1061 to a density of 100 plaques cm⁻². X-Gal was added to the top agar (40 µg ml⁻¹) and recombinant phages, expressing β -D-galactosidase, were detected by the blue colour of plaques.

Transposon mutagenesis with Tn1000. This was done as described by Guyer (1978). Transposon insertion sites were located by isolating plasmid DNA and restricting separately with *Eco*RI, *Bam*HI and *Hind*III.

Southern blot hybridization. *S. bovis* DNA, digested to completion with *Hind*III and *Eco*RI, was fractionated by agarose gel electrophoresis and transferred to nitrocellulose filters (Southern, 1975). DNA probes were labelled by nick-translation (Rigby *et al.*, 1977) to a specific activity of 1–4 × 10⁸ c.p.m. (µg DNA)⁻¹. Conditions for hybridization and subsequent washing of filters were as described by Maniatis *et al.* (1982).

General recombinant DNA procedures. Restriction endonuclease and T4 DNA ligase were used according to the manufacturer's instructions. Transformation of *E. coli* was as described by Cohen *et al.* (1972). DNA was electrophoresed in agarose gels (0.8%–1.5%) using a Tris/borate/EDTA buffer (Meyers *et al.*, 1976). λ DNA digested with *Hind*III, or *Hind*III and *Eco*RI, was used as a molecular size standard. DNA sequencing was done as described by Sanger *et al.* (1980).

Construction of pHG8. A 3 kb *Hind*III/*Kpn*I restriction fragment carrying the *S. bovis lacI* gene was purified from pHG1 and the 5' and 3' protruding termini blunt-ended with T4 polymerase in the presence of all four deoxynucleotide triphosphates. The resultant DNA was cloned into the *Eco*RV site of pACYC184 to generate pHG8.

Enzyme and protein assays. β -D-Galactosidase, lactose permease and thiogalactoside transacetylase were assayed by the method of Miller (1972). β -D-Phosphogalactosidase was determined by the method of Maeda & Gasson (1986). One unit of enzyme activity converts 1 nmol of substrate to product min⁻¹. Protein was measured by the Lowry method.

RESULTS

Detection of β -D-galactosidase and β -D-phosphogalactosidase in S. bovis

S. bovis, grown on minimal medium with lactose as sole carbon source, was assayed for β -D-galactosidase and β -D-phosphogalactosidase. Only the former enzyme activity was detected (Table 2). To investigate whether β -D-galactosidase was induced by β -D-galactosides, *S. bovis* was grown on glycerol minimal medium to early stationary phase, transferred to media containing potential inducers and repressors of lactose catabolism, and assayed for β -D-galactosidase. The results (Table 2) showed that little β -D-galactosidase activity was detected in the absence of β -D-galactosides. Isopropyl thio- β -galactoside (IPTG) was a stronger inducer of the enzyme than lactose. Glucose, but not glycerol, repressed β -D-galactosidase synthesis.

Table 2. *β -D-Galactosidase and phospho- β -D-galactosidase in S. bovis*

Cells of *S. bovis*, grown to early stationary phase in glycerol minimal medium, were transferred to media supplemented with various metabolites. After 3 h, cells were harvested and lysed as described in Methods; DNA was degraded with DNAase and bacterial debris removed by centrifugation at 15000 g for 5 min. Enzymes were assayed as described in Methods.

Addition to growth medium	β -D-Galactosidase [Units (mg protein) ⁻¹]	β -D-Phosphogalactosidase [Units (mg protein) ⁻¹]
None	17	0
Lactose (0.2%)	250	0
IPTG (1 mM)	3300	0
IPTG (1 mM) + glucose (0.2%)	910	0

Table 3. *Expression of S. bovis lac genes in E. coli*

Plasmids were inserted into *E. coli* MC1061. The growth of recombinant strains and preparation of cell-free extracts were as described by Gilbert *et al.* (1986). The *lac* gene proteins were assayed as described in Methods. *E. coli* strain W3110 was used as a control to determine glucose repression of the *E. coli lac* operon. ND, Not determined.

Plasmid	Addition to growth medium	β -D-Galactosidase [Units (mg protein) ⁻¹]	Lactose permease [Units (mg protein) ⁻¹]	Thiogalactoside transacetylase [Units (mg protein) ⁻¹]
pUC19	IPTG	0	0	0
pBR322	IPTG	0	0	0
pHG1	-	22	2.4	1.1
pHG1	Lactose	300	91	43
pHG1	IPTG	3320	1060	680
pHG1	IPTG + glucose	4010	ND	ND
pHG2	-	18	1.1	0.7
pHG2	IPTG	4130	1230	640
pHG3	IPTG	3670	920	0
pHG4	IPTG	0	0	0
pHG5	IPTG	0	0	0
pHG6	IPTG	4360	0	0
pHG7	IPTG	0	0	0
pHG9	-	5100	1700	730
pHG9	IPTG	5250	1790	800
pHG8/pHG9	-	19	2.1	0.2
pHG8/pHG9	IPTG	2820	720	310
pHG8	IPTG	0	0	0
-	IPTG	45000	ND	ND
-	IPTG + glucose	8500	ND	ND

Isolation of the S. bovis lac genes

A gene library of *S. bovis* DNA was constructed in bacteriophage λ 47.1 and a total of 11 000 recombinants were isolated. The recombinant gene bank was screened as described in Methods for phages which synthesized *S. bovis* β -D-galactosidase. Approximately 35 phages were shown to degrade X-Gal. DNA was extracted from five of these phages and digested with various restriction enzymes. A 7.8 kb *Hind*III DNA fragment, common to four of the five phages, was subcloned into the *Hind*III site of pBR322 in both orientations to yield recombinant plasmids pHG1 and pHG2. Cells of *E. coli* MC1061 harbouring either plasmid synthesized *S. bovis* β -D-galactosidase, lactose permease and thiogalactoside transacetylase (Table 3) as well as growing on minimal media containing lactose as sole carbon source. The cleavage sites of different restriction enzymes were mapped within the *S. bovis* DNA present in pHG1 (Fig. 1).

Subcloning and orientation of the lac genes

The positions of the *lac* genes within the 7.8 kb *Hind*III fragment were determined by a combination of transposon mutagenesis and the deletion and subcloning of various restriction fragments into pUC19, followed by determination of β -D-galactosidase, lactose permease and thiogalactoside transacetylase activities (Fig. 1). Partial digestion, with *Eco*RI, and religation of pHG1, generated plasmids pHG3, pHG4 and pHG5. No functional *lac* genes were present in pHG4 and pHG5. Recombinant plasmid pHG3 contained functional β -D-galactosidase and lactose permease genes. When the *Hind*III-*Pst*I (0-6.5) fragment was subcloned into pUC19, cells of MC1061 carrying the recombinant plasmid (pHG6) produced β -D-galactosidase but no lactose permease or thiogalactoside transacetylase. When the *Kpn*I-*Hind*III (3.0-7.8) fragment was subcloned into pUC19 the resultant recombinant plasmid, pHG7, did not contain any functional *lac* genes. Transposon mutagenesis between map positions 0-2.3 did not inactivate any of the *lac* genes, while *Tn*1000 insertions between map position 2.7 and the *Eco*RI (5.5) restriction site inactivated all three genes. Mutations of sequences between map positions 5.7 and 6.6 inactivated the lactose permease and thiogalactoside transacetylase genes. Transposons

Fig. 1. Restriction, deletion and transposon mutagenesis map of *S. bovis* DNA cloned in pHG1. The cleavage sites of restriction enzymes *Eco*RI, *Hind*III, *Pst*I, *Xho*I, *Sst*I, *Pvu*II and *Kpn*I are shown. The following enzymes did not cleave the DNA: *Bam*HI, *Sal*I, *Xba*I, *Xma*I, *Bgl*II and *Sca*I. ○, *Tn*1000 transposon insertion sites. ▨, Regions of deleted *S. bovis* DNA in plasmids derived from pHG1 – the enzyme activities which these plasmids confer on *E. coli* MC1061 are shown.

Fig. 2. Derivation of hybridization probes. The *Pvu*II and *Pst*I cleavage sites in the *E. coli* *lac* operon were determined by Chaconas *et al.* (1981). Probe A was obtained from pNM52 (Gilbert *et al.*, 1986) while probes B and C were derived from pNM480 (Minton, 1984).

inserted into DNA between the *Eco*RI (6·8) and *Hind*III (7·8) restriction sites only inactivated the transacetylase gene.

The above data locate the β -D-galactosidase gene in a region between map positions 2·3 and 5·7, the lactose permease gene between map positions 5·5 and 6·8 and the transacetylase gene between the *Eco*RI (6·8) and *Hind*III (7·8) restriction sites. Removal or mutagenesis of the β -D-galactosidase gene inactivates the other two *lac* genes. Interruption of the lactose permease gene has a polar effect on the transacetylase gene. Alteration of the latter sequence does not reduce expression of the other *lac* genes. These data indicate that the *lac* genes are under operon control with the direction of transcription running from the β -D-galactosidase locus to the transacetylase gene.

Homology of *S. bovis* and *E. coli* lac genes

Homology studies were done, using *Pvu*II and *Pvu*II/*Pst*I DNA restriction fragments from the *E. coli* operon as probes. The derivation of probes A, B and C is shown in Fig. 2. Southern hybridization of the three probes to *Hind*III and *Eco*RI digests of *S. bovis* DNA (Fig. 3) showed that the *E. coli* *lacZ* gene hybridized (even at high stringency) to 7·8 kb *Hind*III and 5·5 kb *Eco*RI DNA restriction fragments. Neither of the other probes hybridized to *S. bovis* DNA under the conditions used. Thus, the *E. coli* *lacZ* gene shows homology to the corresponding *S. bovis* gene.

Regulation of *S. bovis* lac genes in *E. coli*

To determine whether the *S. bovis* *lac* operon was induced in *E. coli*, cells of MC1061 harbouring pHG1 were grown in medium containing β -D-galactosides and lactose catabolic activities determined. Results (Table 3) showed that in the presence of lactose and IPTG there was a 20-fold and 200-fold increase, respectively, of the *lac* gene products. Derivatives of pHG1, containing *Tn*1000 insertions between map positions 1·0 and 2·3, when introduced into MC1061 resulted in the constitutive synthesis of *lac* gene products. These data indicate that a region of DNA upstream of the β -D-galactosidase gene plays an important role in repressing the *lac* operon. To determine whether this locus was *cis* or *trans* acting, the plasmid pHG8 (a multicopy plasmid carrying the 3 kb *Kpn*I–*Hind*III fragment of pHG1, which is compatible with vectors

Fig. 3. Hybridization of *E. coli* *lac* operon DNA probes to *S. bovis* DNA. Chromosomal *S. bovis* DNA, digested with *Hind*III (1) and *Eco*RI (2), were transferred from agarose gels to nitrocellulose filters and hybridized to 32 P-labelled probe A (*lacI*), probe B (*lacZ*) and probe C (*lac Y/A*). After washing at high stringency [$0.1 \times$ SSC (Maniatis *et al.*, 1982) at 65°C] the filters were exposed to X-ray film at -70°C for 24 h. The size of DNA which hybridized to probe B was determined using *Hind*III digests of λ as standards.

utilizing a ColE1 replicon) was constructed, as described in Methods, and introduced into MC1061 carrying pHG9 (pHG1 with *Tn1000* inserted in map position 1.5). Cells containing both plasmids only produced β -galactosidase in the presence of IPTG. Thus, the DNA region between map positions 1 and 2.7 encodes a protein which is *trans* acting and represses the *S. bovis* *lac* operon in the absence of IPTG or lactose.

Production of β -D-galactosidase in *S. bovis* is repressed by glucose. The regulation of the cloned gene was therefore investigated using *E. coli* MC1061 containing pHG1. Wild-type *E. coli* W3110 was used as a control. Cells were cultured in L-broth with and without glucose, and β -D-galactosidase activity monitored (Table 3). Although the *E. coli* *lacZ* gene was repressed by glucose, the metabolite had no effect on the corresponding *S. bovis* gene.

Interspecies regulation of the E. coli and S. bovis lac operons

The interaction of the *E. coli* repressor protein with the *S. bovis* operator was investigated. *E. coli* MC1061 containing the *E. coli* *lacI* gene, encoded by pNM52 (Gilbert *et al.*, 1986), and pHG9, which contains the *S. bovis* *lac* operon but an inactive *lacI* gene, was grown in the presence and absence of IPTG and β -D-galactosidase activity determined. The results (Table 4) showed that the *lac* operon was induced 200-fold by IPTG. The binding of the *S. bovis* *lacI* gene product to the *E. coli* *lac* operon was studied in a *lacI* strain of *E. coli* (CA159) harbouring pHG8 (contains the *S. bovis* *lacI* gene). β -D-Galactosidase was produced constitutively in CA159 but when the strain harboured pHG8 there was a 1000-fold induction of the enzyme in the presence of IPTG. Thus, the *lac* repressor protein from both bacteria shows interspecies activity.

Isolation and nucleotide sequence of the S. bovis lac promoter

The 4.5 kb *Sst*I-*Hind*III DNA restriction fragment was digested with *Taq*I, cloned into the *Acc*I site of the promoter-probe vectors pNM480, pNM481 and pNM482, (Minton, 1984) and transformed into MC1061. Promotor activity was detected by the ability of recombinants to hydrolyse X-Gal. Promotor activity was detected when *Taq*I DNA restriction fragments were

Recombinant plasmids were inserted into *E. coli* MC1061 or CA159. The growth of recombinant strains, preparation of cell-free extracts and assay of β -D-galactosidase were as described in Table 3.

The *S. bovis* *lac* regulatory sequence was removed from pNM480 by digestion with *Hind*III and *Eco*RI, inserted into M13mp8 and M13mp9, and sequenced by the dideoxy chain termination method of Sanger *et al.* (1980). The positions of putative -10 and -35 promoter regions, operator and Shine-Dalgarno sequences, together with the corresponding *E. coli* regions, are shown in Fig. 4. There was strong homology between the operator sequences and the 5' sequences of the *lacZ* structural genes (Kalnins *et al.*, 1983). Little similarity between the -10 and -35 promoter regions was observed, and the CAP binding sequence of *E. coli* was absent in *S. bovis* DNA. The putative Shine-Dalgarno region of *S. bovis* was similar to the consensus ribosome binding sequence of Gram-positive bacterial mRNA (McLaughlin *et al.*, 1981).

DISCUSSION

The *S. bovis lac* genes were cloned and expressed in *E. coli*. Initially, the genes were located in a 7.8 kb *Hind*III restriction fragment, which was cloned into pBR322 in both orientations to give the recombinant plasmids pHG1 and pHG2. *E. coli* harbouring either plasmid produced similar levels of *lac* catabolic proteins, indicating that the promoter region of the genes had also been cloned. The level of expression of the β -D-galactosidase gene in *E. coli* and *S. bovis* was similar. As there were 30–50 copies of pHG1 in *E. coli* (data not shown) and only a single copy of the *lacZ* gene in *S. bovis*, it appears that the *streptococcal* promoter is not efficiently utilized in *E. coli*.

Data presented in this report showed that the three *lac* proteins were present in a constant ratio regardless of bacterial growth conditions. Transposon mutagenesis or deletion of the β -D-galactosidase gene had a polar effect on the two other *lac* genes, while alteration of the *lac* permease gene only inactivated the transacetylase gene. Removal of the latter gene had no effect on β -galactosidase or lactose permease expression. The three *lac* proteins were induced by IPTG, but were synthesized constitutively when DNA sequences adjacent to the *lac* genes were altered. Thus, the three *lac* genes constitute an operon which is subject to negative control by a repressor protein whose gene is closely linked to the operon. β -D-Galactopyranosides inactivate the repressor protein; hence they induce synthesis of the Lac proteins. The levels of induction by IPTG (200-fold) of the *S. bovis lac* operon, either in *E. coli* or *S. bovis*, were similar. Thus, the *lac* repressor gene is expressed efficiently in *E. coli*. In contrast to this, the *lac* operons from certain enterobacteria are subject to anomalous expression in heterologous enterobacterial hosts because of low levels of the *lacI* protein (Baumberg & Roberts, 1984; Baumberg *et al.*, 1980).

The putative *S. bovis lac* promoter and operator has been cloned, sequenced and compared with the corresponding *E. coli* region. The putative *S. bovis* operator was very similar to the *E. coli lacZ_o* sequence. This provides an explanation for the strong interspecies activities of the *lac* repressor proteins from the two bacteria. That the CAP binding sequence present in *E. coli lacZ_p* was absent from the *S. bovis* promoter explains the lack of glucose repression of the streptococcal *lac* operon in *E. coli*. This is consistent with the observation that most Gram-positive bacteria do not synthesize cAMP (Lee *et al.*, 1982). The mechanism of glucose repression exerted on the *lac* operon in *S. bovis* is unknown.

The homology between the *lacZ* genes, and the similarity in the organization and regulation of the *lac* operons of the two bacteria, is surprising, as *S. bovis* is a Gram-positive rumen anaerobe, while *E. coli* is a Gram-negative enteric aerobe. Two possible explanations for these observations are: (i) the *lac* operon was transferred from an enteric bacteria to *S. bovis* via a transposon. The *lac* genes from the enteric bacterium *Yersinia enterocolitica* (Cornelis *et al.*, 1978) are encoded by Tn951; (ii) *S. bovis* inherited the lactose catabolic pathway from enterobacterial plasmids such as those found in the genus *Klebsiella* (Brenchley & Magasanik, 1972).

Future work in this laboratory will utilize the cloned *S. bovis lac* regulatory region to construct highly regulated expression vectors for this rumen micro-organism.

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