J. Mol. Microbiol. Biotechnol. (2000) 2(4): 483-490.

**JMMB Review**

# Regulation of the *pts* Operon in Low G+C Gram-Positive Bacteria

**Christian Vadeboncoeur*, Michel Frenette, and Louis-André Lortie**

Groupe de recherche en écologie buccale (GREB), Département de biochimie et de microbiologie, Faculté des sciences et de génie, and Faculté de médecine dentaire, Université Laval, Sainte-Foy, Québec, Canada, G1K 7P4

*“Toute notre dignité consiste donc en la pensée. C’est de là qu’il nous faut relever et non de l’espace et de la durée, que nous ne saurions remplir. Travaillons donc à bien penser: voilà le principe de la morale” (Blaise Pascal)*

## Abstract

**The sugar transport system called phosphoenolpyruvate: sugar phosphotransferase (PTS) is widespread among eubacteria. Its is generally composed of two cytoplasmic proteins, HPr and EI, which are found in all bacteria possessing a PTS, and a family of EIIs whose number, specificity, and molecular structure in terms of domain arrangement vary from species to species. In low G+C Gram-positive bacteria, the genes coding for the general proteins HPr and EI, designated *ptsH* and *ptsI* respectively, are organized into the *pts* operon. In this paper, we summarize current knowledge about the regulation of the *pts* operon in low G+C Gram-positive bacteria. Physiological data indicate that EI and most particularly HPr make up a substantial proportion of cellular proteins. Their synthesis is not coordinated and is influenced by environmental factors. The principal DNA *cis*-elements involved in the regulation of *pts* operon transcription are a strong promoter whose sequence and structure are very similar to those of the canonical promoter recognized by the *Escherichia coli* and *Bacillus subtilis* major RNA polymerases, a 5'-untranslated region, a rho-dependent terminator located at the 5' end of *ptsI*, and an intrinsic terminator located downstream from *ptsI*. Analysis of *ptsH* and *ptsI* Shine-Dalgarno sequences as well as experimental results obtained with a *Streptococcus salivarius* mutant suggest that the expression of HPr and EI is also controlled at the translation level.**

## Introduction

The phosphoenolpyruvate: sugar phosphotransferase system (PTS) is involved in the transport and phosphorylation of several mono- and disaccharides as well as multiple regulation mechanisms (Postma *et al.*, 1993; Saier and Reizer, 1994; Saier *et al.*, 1996). These functions allow the cell to adjust its physiology and biochemistry with respect to the availability of carbon and energy sources. PTS proteins are commonly divided into two groups on the basis of their general or specific participation in the transport of sugars ie., the general energy coupling proteins EI and HPr, and a family of soluble or membrane proteins referred to as Enzyme IIs (EII). The role of the energy coupling proteins is to produce, at the expense of PEP, the eclectic intermediate HPr(His~P), which possesses the property to transfer its phosphate group to several classes of proteins, including all the EII complexes. The EIIs, which are composed of at least three domains called A, B, and C, serve two purposes with respect to sugar acquisition: to transport, in a more or less specific manner, sugars across the cytoplasmic membrane and to phosphorylate the incoming sugar (Saier and Reizer, 1992; Postma *et al.*, 1993). In addition to their role in sugar transport, HPr and some EII domains or proteins also possess regulatory functions (Postma *et al.*, 1993; Saier *et al.*, 1996).

The PTS has been detected in a broad variety of bacteria, including Gram-positive bacteria with a DNA base composition of less than 50 mol% guanosine plus cytosine (G+C), a group that constitutes the so-called *Clostridium* branch. Several members of this subphylum, including *Bacillus*, *Enterococcus*, *Staphylococcus*, *Streptococcus*, *Lactococcus*, *Lactobacillus*, and *Pediococcus* (Romano *et al.*, 1970; Romano *et al.*, 1979), *Clostridium* (Mitchell *et al*, 1995), and *Listeria* (Parker and Hutkins, 1997) have been found to transport one or more sugars via the PTS.

In almost every bacterial species studied so far that possess HPr and EI, the genes encoding these proteins, designated *ptsH* and *ptsI* respectively, are organized into the *pts* operon. The objective of this paper is to review the factors that control the expression of the *pts* operon in low G+C Gram positive bacteria.

## Physiological Data

### About the Cellular Amount of HPr

A striking feature observed by several authors is the surprising abundance of cellular HPr protein. The cellular concentration in *Streptococcus pyogenes* cells has been estimated at 0.16 mM (Reizer *et al*, 1984). Thibault and Vadeboncoeur (1985) reported that HPr represents roughly 5% of total cell protein in various strains of *Streptococcus mutans*. Values of the same magnitude have also been reported for *Streptococcus sanguis* and *Streptococcus salivarius*, with HPr representing approximately 3.3% to 7% of all cell proteins (Jenkinson, 1989; Gauthier *et al.* 1990). However, these values must be revised as it was later found that the classic techniques used to determine protein concentrations, such as the Lowry and Bradford assays, overestimated the amount of streptococcal HPr two- to six fold respectively (Gauthier *et al.*, 1997). The proportion of HPr with respect to total cellular protein in oral streptococci may thus be closer to 2% to 4%, which

*For correspondence. Email Christian.Vadeboncoeur@bcm.ulaval.ca; Tel. 418-656-2319; Fax. 418-656-2861.

© 2000 Horizon Scientific Press

still represents a relatively high percentage. Assuming that the intracellular volume is 2.3µl/mg of dry cells (Hamilton and Buckley, 1991) and that proteins account for half of the cellular mass, the cellular concentrations of HPr in oral streptococci would range from 0.5 to 1 mM.

The high level of HPr in cells raises the question as to whether such quantities are necessary for optimal sugar transport by the PTS. This situation appears all the more disconcerting since the Km of EI for HPr ranges from 25 µM to 44 µM (Vadeboncoeur *et al.*, 1983; Kohlbrecher *et al.*, 1992), while that of *Bacillus* $IIA^{Glc}$ for HPr(His~P) is 0.5 µM (Reizer *et al.*, 1992). We recently isolated *S. salivarius* mutants with point mutations in the promoter region of the *pts* operon and in the Shine-Dalgarno sequence of *ptsH* that resulted in a reduction in the total amount of cellular HPr (Thomas *et al.*, 1997). Analysis of these mutants indicated that a reduction of EI and total HPr by a factor two does not affect growth on the PTS sugars fructose and glucose. As EI negative mutants of *S. salivarius* do not grow on these sugars (Gauthier *et al*, 1994), the presence of non-PTS transport systems for glucose and fructose is unlikely in *S. salivarius*. Therefore, results obtained with the promoter PTS mutants suggest that at least half of the cellular HPr is not required for optimal functioning of the PTS and is obviously synthesized to accomplish other functions, an assumption supported by an impressive array of data demonstrating the involvement of HPr(Ser-P) and HPr(His~P) in carbon catabolite repression and activation in Gram-positive bacteria (Saier *et al.*, 1996; Stülke *et al.*, 1998). The regulation of the expression of the *pts* operon is thus dictated by two cellular imperatives: the need to ensure an optimal provisioning of carbon and energy sources and the need to maintain an appropriate equilibrium between various metabolic pathways to ensure optimal growth.

**About the HPr/EI Ratio**

As HPr is the substrate of at least two enzymes, EI and HPr(Ser) kinase-phosphatase, and since HPr has to interact with several other proteins to accomplish its multiple functions, whereas EI catalyzes a single reaction, one might expect that cells would contain higher amounts of HPr than EI. Studies conducted with oral streptococci have indeed indicated that cells synthesize much more HPr than EI. The HPr/EI molar ratio in various strains of *S. mutans* varies from 5 to 7 (Thibault and Vadeboncoeur, 1985), and a ratio of 100 has been reported in *S. salivarius*. (Gauthier *et al.*, 1990). However, at the time these values were calculated, the amount of HPr in reference solutions was determined by the Lowry method, which as mentioned above gives erroneous data. Hence, if we take into account the error introduced by measuring HPr using the Lowry assay, the HPr/EI ratio in *S. mutans* should vary from 2 to 3, and in *S. salivarius* from 30 to 50. The difference in the HPr/EI ratios between these two microorganisms appears to be caused mainly by the fact that *S. mutans* produces more EI (approximately 0.8 to 1 nmol/mg of cell protein) (Thibault and Vadeboncoeur, 1985) than *S. salivarius* (about 0.1 nmol/mg of cell protein) (Gauthier *et al.*, 1990). To our knowledge, the intracellular amounts of HPr and EI have never been measured in other low G+C Gram positive-bacteria, raising questions about whether the findings obtained with oral streptococci apply to other members of the *Clostridium* subphylum. However, as a difference in the expression of HPr and EI has also been observed in the Gram-negative bacteria *Escherichia coli* and *Salmonella typhimurium* with HPr/EI ratios ranging from 6 to 25 (Mattoo and Waygood, 1983), it is reasonable to believe that the uncoordinated expression of EI and HPr is a common trait among bacteria possessing a *pts* operon.

**About the Influence of Environmental Conditions**

The effect of environmental conditions on cellular levels of HPr and EI was first studied using batch grown cells. Studies conducted with *S. sobrinus* ATCC 27352 (formerly *S. mutans*) indicate that cellular levels of these proteins vary two-fold at the most as a function of the growth sugar (Thibault and Vadeboncoeur, 1985). Recent data has revealed that the levels of total HPr in *S. salivarius* are almost the same in cells grown in glucose (PTS sugar), or lactose or galactose (non-PTS sugars) (Gauthier *et al.*, 1997; Plamondon *et al.*, 1999), suggesting that the *pts* operon is expressed at virtually the same level whether the cells are growing at the expense of a PTS or a non-PTS sugar. Similar results have been reported for *E. coli* and *S. typhimurium* (Mattoo and Waygood, 1983). Finally, expression of the *pts* operon of *Bacillus stearothermophilus* cloned in pUC18 and expressed in *E. coli*, has been reported to increase with temperature, the levels of HPr and EI being five-fold higher in cells grown at 37°C than in cells grown at 30°C (Lai and Ingram, 1995). Unfortunately, the results have not been confirmed by experiments conducted with *B. stearothermophilus*.

Studies conducted with streptococcal cells grown in continuous culture have, however, provide convincing evidence that the expression of the *pts* operon responds to environmental conditions in a complex manner. Earlier studies carried out with *S. mutans* and *S. sobrinus* suggest that HPr concentrations are only slightly influenced by growth rate, growth at varying pH, and glucose concentration (Vadeboncoeur *et al.*, 1987; Rodrigue *et al.*, 1988; Vadeboncoeur *et al.*, 1991). In these studies, however, the cellular amounts of HPr were determined by rocket immunoelectrophoresis, a technique that does not allow separation of the chemically different forms of HPr. In a more recent study (Thevenot *et al.*, 1995), the intracellular levels of HPr were determined in *S. mutans* Ingbritt by crossed immunoelectrophoresis, a technique that allows the total amount of HPr to be calculated from the amount of each form (Vadeboncoeur *et al.*, 1991). Using this technique, it was shown that increasing the glucose concentration from 10 mM to 200 mM caused a two-fold increase in the total amount of HPr in cells grown at pH 7.0 and a dilution rate of 0.1 $h^{-1}$.

Growth conditions also affect the synthesis of EI in *S. mutans*. Work with *S. mutans* DR0001 cultured in a chemostat at different dilution rates, pH and glucose concentrations, showed that cellular levels of EI are highest in cells grown under conditions of glucose limitation at a dilution rate of 0.40 $h^{-1}$ and pH 7.0, and five-fold lower in cells cultured under conditions of glucose excess at the same rate and pH (Rodrigue *et al.*, 1988). The effect of glucose concentration on the cellular amount of EI was investigated in a more comprehensive study conducted with *S. mutans* Ingbritt where cells were cultured at eight glucose concentrations from 2.6 mM to 304 mM, at a dilution rate of 0.1 $h^{-1}$ and pH 7.0 (Hamilton *et al.*, 1989). The amount of EI dropped at all glucose levels above 2.6

mM (glucose limitation) to a maximum of four-fold at 304 mM (glucose excess). Taken together, these results indicate that the intracellular amounts of HPr and EI vary in opposite directions with respect to glucose availability. Simple calculations reveal that the HPr/EI molar ratio in *S. mutans* Ingbritt grown in continuous culture is approximately 6 under glucose limited conditions and rises to about 25 under glucose excess conditions. These values were calculated by taking into account the two-fold overestimation that occurs when the amount of HPr is measured with the Lowry assay.

**Physiological Data: Conclusion**

Information obtained from measurements of the intracellular levels of the PTS general energy coupling proteins in low G+C Gram positive bacteria can be summarized as follows: (1) cells synthesize very high amounts of HPr, suggesting that the encoding gene is under the control of a strong promoter; (2) cells synthesize much more HPr than EI, suggesting that transcription of *ptsH* and *ptsI* is uncoupled; (3) the ratio HPr/EI is not the same from one species to the other; (4) the synthesis of HPr and EI as well as the HPr/EI ratio are influenced by environmental conditions; (5) HPr and EI are always expressed at relatively high levels irrespective of the nature of the sugar that sustains growth. These data suggest that cellular levels of HPr and EI are maintained at appropriate levels through complex regulatory mechanisms. The next section describes the prominent *cis* elements governing transcription of the *pts* operon in low G+C Gram-positive bacteria as well as the results of transcriptional analysis.

## Transcriptional Data

**About the Composition of the *pts* Operon**

In low G+C Gram-positive eubacteria, the *pts* operon is generally composed of two genes, *ptsH* and *ptsI*, coding respectively for the general energy coupling proteins HPr and Enzyme I. Two exceptions to this rule have been identified: (1) a gene designated *ptsT*, which is located downstream from *ptsI* is co-transcribed with *ptsH* and *ptsI* in *B. stearothermophilus* (Lai and Ingram, 1995). The function of PtsT and its link with the PTS remain to be demonstrated; (2) in *B. subtilis*, the transcription of *ptsH* and *ptsI* is also under the control of a common promoter. However, when cells are grown on glucose, the *ptsG* gene, which is located upstream from the *pts* operon and coding for the protein $IIABC^{Glc}$, is cotranscribed with *ptsH* and *ptsI*. Thus, the *ptsH* and *ptsI* of *B. subtilis* are part of two overlapping operons, the *pts* operon and the *ptsGHI* operon (Stülke *et al*., 1997).

**About the *cis* Elements Controlling Transcription of the *pts* Operon**

Transcription of the *pts* operon has been studied in *S. salivarius* (Gagnon *et al*., 1995), *Lactobacillus sake* (Stentz *et al*., 1997), *Listeria monocytogenes* (Christensen *et al*., 1998), *Lactococcus lactis* (Luesink *et al*., 1999), *B. subtilis* (Stülke *et al*., 1997), *Streptococcus thermophilus* (Cochu *et al*., 1999), *S. mutans* (Daigle, Vadeboncoeur, and Frenette, unpublished results), and *Lactobacillus casei* (Viana *et al*., 2000). The *cis*-elements involved in the transcription of the *pts* operon identified from these studies are a promoter located upstream from *ptsH*, a 5'-untranslated region, a rho-dependent type terminator located at the 5' end of *ptsI*, and an intrinsic terminator located downstream from *ptsI* (Figure 1).

**About the Promoter of the *pts* Operon**

In low G+C Gram-positive bacteria, the *pts*-specific mRNAs originate from a single promoter located upstream from the *ptsH* gene. The precise location of the promoter was determined by primer extension experiments in *S. salivarius* (Gagnon *et al*., 1995), *Lb. sake* (Stentz *et al*., 1997), *Lc. lactis* (Luesink *et al*., 1999), *Ls. monocytogenes* (Christensen *et al*., 1998), and *B. subtilis* (Gonzy-Treboul *et al*., 1989) . The analysis of the *B. subtilis pts* promoter was performed in *E. coli* and the results were different from those produced by the *B. subtilis* genome sequencing project. Since the promoter sequence determined by the genome sequencing project is closer to the sequence of the other *pts* promoters, it will be used in this paper for sequence comparisons. Figure 2A illustrates several features of the *pts* promoter identified either experimentally or from *in silico* analysis. Most *pts* promoters possess a conserved TTG sequence at the 5'-end of the –35 box. Only *B. subtilis* and *Bacillus megaterium pts* promoters differ slightly, having ATG and TTT sequences respectively. TTG is the sequence found at the 5' end of the -35 hexamer of the canonical promoter recognized by the major *E. coli* RNA polymerase and the principal form of the *B. subtilis* RNA polymerase. These nucleotides are the most highly

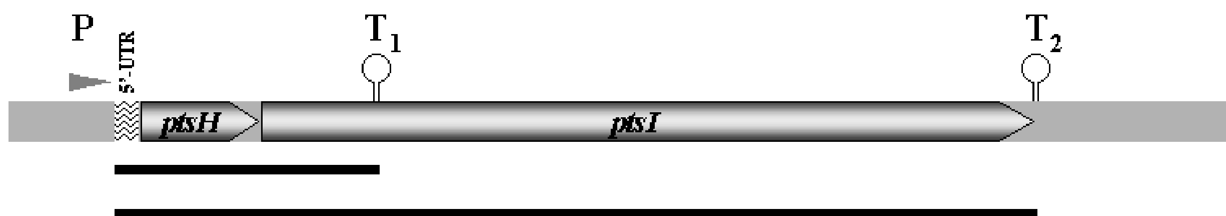


Figure 1. Schematic representation of the *pts* operon of low G+C Gram-positive bacteria and *pts*-specific transcripts. The operon is composed of two genes, *ptsH* and *ptsI*, which code for the PTS proteins HPr and EI respectively. The principal *cis* elements involved in transcription regulation are a single promoter (P) located upstream from *ptsH*, a 5'-untranslated region (5'-UTR) located between the transcription initiation site and the translation initiating codon of *ptsH*, a rho-dependent terminator (T1) located at the 5'-end of *ptsI* (not found in all *pts* operons), and an intrinsic terminator (T2). A long transcript encompassing *ptsH* and *ptsI* that terminates at T2 has been detected in all species studied so far. In several species, an additional small transcript specific to *ptsH* that terminates at T1 has also been detected.

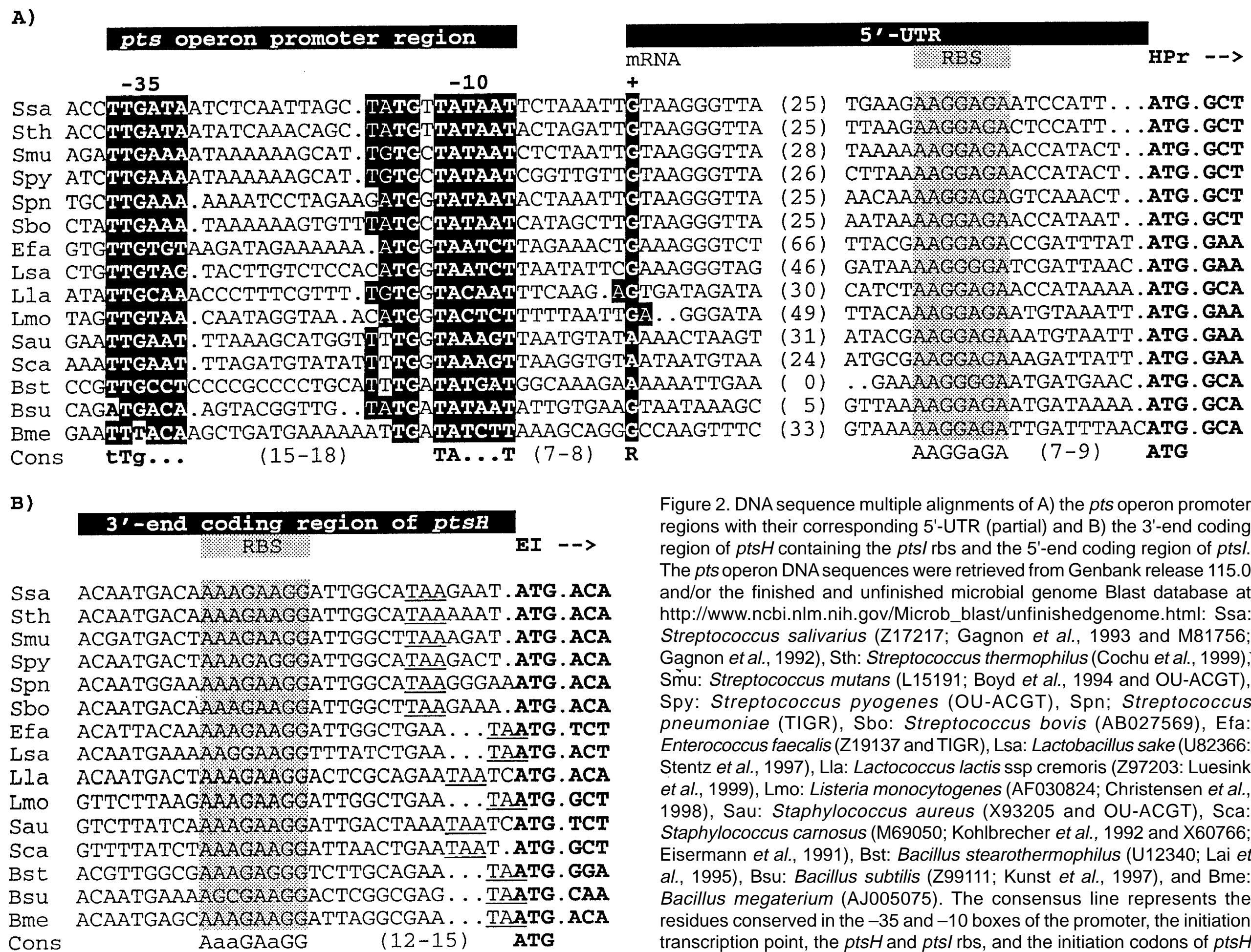


Figure 2. DNA sequence multiple alignments of A) the *pts* operon promoter regions with their corresponding 5'-UTR (partial) and B) the 3'-end coding region of *ptsH* containing the *ptsI* rbs and the 5'-end coding region of *ptsI*. The *pts* operon DNA sequences were retrieved from Genbank release 115.0 and/or the finished and unfinished microbial genome Blast database at http://www.ncbi.nlm.nih.gov/Microb_blast/unfinishedgenome.html: Ssa: *Streptococcus salivarius* (Z17217; Gagnon *et al.*, 1993 and M81756; Gagnon *et al.*, 1992), Sth: *Streptococcus thermophilus* (Cochu *et al.*, 1999), Smu: *Streptococcus mutans* (L15191; Boyd *et al.*, 1994 and OU-ACGT), Spy: *Streptococcus pyogenes* (OU-ACGT), Spn; *Streptococcus pneumoniae* (TIGR), Sbo: *Streptococcus bovis* (AB027569), Efa: *Enterococcus faecalis* (Z19137 and TIGR), Lsa: *Lactobacillus sake* (U82366: Stentz *et al.*, 1997), Lla: *Lactococcus lactis* ssp cremoris (Z97203: Luesink *et al.*, 1999), Lmo: *Listeria monocytogenes* (AF030824; Christensen *et al.*, 1998), Sau: *Staphylococcus aureus* (X93205 and OU-ACGT), Sca: *Staphylococcus carnosus* (M69050; Kohlbrecher *et al.,* 1992 and X60766; Eisermann *et al.*, 1991), Bst: *Bacillus stearothermophilus* (U12340; Lai *et al.*, 1995), Bsu: *Bacillus subtilis* (Z99111; Kunst *et al.*, 1997), and Bme: *Bacillus megaterium* (AJ005075). The consensus line represents the residues conserved in the –35 and –10 boxes of the promoter, the initiation transcription point, the *ptsH* and *ptsI* rbs, and the initiation codons of *ptsH* and *ptsI*. A upper case letter indicates that the nucleotide is conserved in all sequences, whereas a lower case letter indicates that it is conserved in at least 13 of 15 sequences.

conserved in the –35 consensus sequence. The next three nucleotides of the –35 box of the *pts* promoters are less well conserved. However, several –35 hexamers of the *pts* promoter listed in Figure 2A differ by only one nucleotide from the canonical –35 hexamer (TTGACA). The two first nucleotides (TA) at the 5' end of the –10 box and the last nucleotide of the hexamer (T) are universally conserved and correspond to the nucleotides of the canonical –10 box (TATAAT). These three nucleotides are the ones that occur most frequently in the –10 region of promoters recognized by the *E. coli* and *B. subtilis* major RNA polymerases. The sequences of the 10 hexamer of the *pts* promoters of streptococci and *B. subtilis* are identical to the –10 canonical sequence. For the other species, the centre portion of the hexamer is variable, a situation that is frequently observed among strong promoters (Voskuil and Chambliss, 1998). The spacing between the –35 and –10 sequences varies from 15 to 18 nucleotides, a distance considered to be optimal for this type of promoter (Ayers *et al.*, 1989 ; Voskuil *et al.*, 1995). The TRTG motif (-16 region) that was found in several promoters of *B. subtilis* and other Gram-positive bacteria (Voskuil *et al.*, 1995; Voskuil and Chambliss, 1998; Borst and Bentley, 1994; Kenney and Churchward, 1996) is present in several *pts* promoters. This motif was found to significantly influence the strength of the promoter, especially when the –35 region is weakly conserved. However, whether this motif plays a role in the transcription of the *pts* operon in low G+C Gram-positive bacteria remains to be demonstrated. The -10 hexamer of the *pts* promoter of *Ls. monocytogenes* was placed by Christensen *et al.* (1998) one nucleotide upstream from the transcriptional start point. However, comparison of the *Ls. monocytogenes pts* promoter with *pts* promoters from other low G+C Gram-positive eubacteria (Figure 2A) reveals the presence in *Ls. monocytogenes* of a –10 box in a region that would be more consistent with the classic location of this hexamer and would align the TG motif with the TG sequences of the other *pts* promoters.

Further sequence analysis has shown that *pts* streptococcal promoters, but not promoters from other species, possess a catabolite-responsive element (CRE) sequence bearing only one mismatch with the consensus sequence (Miwa *et al.*, 2000). The CRE sequence overlaps the transcription initiation site, suggesting that it may negatively regulate the transcription of the *pts* operon. Further work is required to verify this hypothesis.

**About the 5'-UTR**

The extent of the 5'-untranslated region (5'-UTR) of the transcript located between the transcription initiation site and the translation initiating AUG ranges from 29 to 97 nucleotides. *In silico* analysis has revealed that these 5'-UTRs can form secondary structures. Secondary structures in 5'-UTR regions have been shown to stabilize mRNAs (Coburn and Mackie 1999) and play important roles in the regulation of gene expression (Fang *et al*., 1998). Whether this untranslated mRNA region of the *pts* operon has an effect on the regulation of HPr and EI expression remains to be investigated. Interestingly, we have recently found that a C to A transversion at position +21 of the *pts* 5'-UTR of *S. salivarius* decreases cellular levels of HPr and EI and renders the cell insensitive to the glucose and fructose catabolite repression (Thomas *et al*., 1997).

**About the rho-Dependent Terminator**

Several low G+C Gram positive bacteria possess a rho-dependent terminator at the 5'-end of *ptsI*. The functionality of this structure was demonstrated in *S. salivarius* by S1 nuclease mapping (Gagnon *et al*., 1995) and was inferred from Northern blot analysis in *S. mutans* (Daigle, Vadeboncoeur, and Frenette, unpublished results), *S. thermophilus* (Cochu *et al*., 1999), *Lb. sake* (Stentz *et al*., 1997) and *Lc. Lactis* (Luesink *et al.,* 1999). Dissociation of the polymerase from the DNA at this terminator generates a 0.5 kb *ptsH*-specific mRNA in *S. salivarius*, *S. mutans* and *S. thermophilus*, and a 0.6 kb transcript in *Lb. sake*. Surprisingly, the size of the specific *ptsH* mRNA of *Lc. Lactis* determined by Northern blot analysis is 0.3 kb (Luesink *et al*., 1999) whereas the predicted size of the transcript calculated from the position of the stem-loop structure inferred from *in silico* analysis is 0.5 kb. Since a 0.3 kb transcript is too small to generate a complete HPr protein, the actual size of the transcript is most probably 0.5 kb. The presence of this small *ptsH*-specific transcript in these species indicates that transcription of *ptsH* and *ptsI* is uncoupled, which is consistent with the finding that cells contain higher amounts of HPr than EI. In *S. salivarius*, the terminator that generates the small *pts* transcript has been shown to stop 49% of the transcriptional waves (Gagnon *et al*., 1995). As a consequence, the ratio of the *ptsH*-bearing mRNAs versus those bearing *ptsI* is 2:1. This difference in the relative level of mRNA synthesis partially accounts for the disparities between the intracellular concentrations of HPr and EI (Gagnon *et al*., 1995; Kohlbrecher *et al*., 1992). No small transcript has been detected in *Ls. monocytogenes* (Christensen *et al*., 1998), *Lb. casei* (Viana *et al*., 2000), or *B. subtilis* (Stülke *et al*., 1997). In the latter case, however, the probes used in the Northern blot experiments were not designed to detect the small *ptsH* specific transcript, so its presence in *B. subtilis* remains to be established.

**About the Intrinsic Terminator**

A long transcript encompassing the entire *pts* operon has been detected in all species so far studied. This transcript originates from the promoter located upstream from *ptsH* and terminates at a rho-independent terminator 6 to 31 nucleotides downstream from the 3'-end of *ptsI* (Figure 1). This intrinsic terminator has been identified experimentally or by *in silico* analysis in all *pts* operons so far studied in low G+C Gram-positive bacteria with the exception of *St. carnosus*, which possesses a stem-loop structure at the end of *ptsI* without the run of U residues typical of rho-independent terminators (Kolhbrecher *et al*., 1992). Termination efficiency is 100% in *Lb. sake* (Stentz *et al*., 1997), *Lc. lactis* (Luesink *et al*., 1999), *Ls. monocytogenes* (Christensen *et al*., 1998), *B. subtilis* (Stülke *et al*., 1997), *S. thermophilus* (Cochu *et al*., 1999), and *S. mutans* (Daigle, Vadeboncoeur, and Frenette, unpublished results), confirming that no other gene downstream from *ptsI* is part of the *pts* operon. In *S. salivarius* ATCC 25975, an IS downstream from *ptsI* generates a rho-independent terminator contiguous with the *pts* intrinsic terminator (Gagnon *et al*.,1995 ; Lortie *et al*., 1994). Some 38% of the transcripts pass through the intrinsic terminator but no transcription occurs beyond the IS terminator (Gagnon *et al*., 1995). These results suggest that *gapN*, which is located downstream from *ptsI* (Boyd *et al*., 1995), can be co-transcribed with the *pts* operon in *S. salivarius* (Gagnon *et al*., 1995). However, transcriptional analysis of the *pts* operon in other *S. salivarius* strains that do not possess an IS downstream from *ptsI* indicates that transcription does not go beyond the *pts* intrinsic terminator, indicating that *gapN* is not co-transcribed with *ptsH* and *ptsI* (Daigle, Vadeboncoeur, and Frenette, unpublished results). The idiosyncrasy observed in *S. salivarius* ATCC 25975 is obviously caused by a decrease in termination efficiency at the *pts* intrinsic terminator by the IS.

**About Environmental Conditions**

Physiological analyses have shown that the amount of HPr and EI in batch-grown cells varies at the most two-fold with respect to the type of growth sugar. Transcription analysis of the *pts* operon indicates that growing cells of *B. subtilis* (Gonzy-Treboul *et al*., 1989), *Lb. casei* (Viana *et al*., 2000), and *S. salivarius* (Daigle, Vadeboncoeur, and Frenette, unpublished results) on glucose enhances transcription of the *pts* operon by a factor of two or less, a result that is consistent with the physiological data. Experiments conducted with *Lc. Lactis* revealed a ten-fold difference in the amount of *pts* transcripts between glucose- and galactose-grown cells (Luesink *et al*., 1999). These transcriptional data, however, have not been substantiated by measurements of the cellular levels of HPr and EI. Conversely, transcription of the *pts* operon in *Lb. Sake* is not influenced by the nature of the sugar that sustains growth (Stentz *et al*., 1997). Experiments conducted with *Ls. monocytogenes* indicated that the amount of a transcript encompassing *ptsH* and *ptsI* varies with the amount of glucose remaining in the culture medium and the external pH (Christensen *et al*., 1998). High amounts of transcript were detected in cells harvested when the glucose concentration ranged from 7.4 mM to 73 mM, whereas the transcript was barely detectable at glucose concentrations below 1.2 mM and undetectable when the glucose concentration dropped to 0.4 mM. With respect to external pH, the amount of transcript was high in cells harvested from cultures with pH values of 6.6 to 5.3, dropped when the pH reached 4.4, and became undetectable at pH 4.2. Unfortunately, the results were not confirmed by measurement of HPr and EI cellular concentrations. Moreover, the results were obtained from batch-grown cells harvested at different stages of growth, that is under conditions where several factors such as medium pH, sugar concentration, cellular density, and concentration of

fermentation products were not controlled. The results would have been more conclusive if the experiments had been conducted with cells grown in a chemostat where growth parameters could be controlled.

### Transcriptional Data: Conclusion

Transcription of the *pts* operon in low G+C Gram positive bacteria is under the control of a strong promoter, which is consistent with the abundance of *pts*-specific transcripts and the high levels of HPr and EI detected in growing cells. In all *pts* operons, a 5'UTR of 29 to 97 nucleotides precedes the AUG initiation codon of *ptsH*. Preliminary results obtained with *S. salivarius* suggest that this region is important for optimal expression of the operon. In many species, transcription is partially stopped by a rho-dependent terminator at the 5' end of *ptsI*, resulting in the formation of a short *ptsH*-specific transcript and a longer transcript encompassing *ptsH* and *ptsI*. This transcriptional characteristic may to some extent explain the differences in the levels of HPr and EI found in cells. Some species, however, produce only one transcript encompassing both genes of the operon, indicating that the mechanisms controlling the cellular HPr/EI ratio vary within the *Clostridium* subphyllum. Transcription of the *pts* operon is influenced by environmental conditions, a result which is also consistent with physiological data. However, the results indicate that species react in a specific manner to different external stimuli, suggesting that a variety of mechanisms may be involved in the transcriptional regulation of the *pts* operon among low G+C Gram positive bacteria.

## Translational Control of HPr and EI Synthesis

The structure of the *pts* promoter and the uncoordinated transcription of *ptsH* and *ptsI* observed in several bacterial species are consistent with the high amounts of HPr and EI found in cells and with the fact that these proteins are produced at different levels. Nevertheless, the uncoordinated expression of HPr and EI may also result from translational regulatory mechanisms. Sequence analysis of putative *ptsH* ribosome binding sites (rbs) of low G+C Gram-positive bacteria reveals a high degree of similarity with the optimal rbs sequence of *B. subtilis* (AAGGaGA) (Figure 2A) (Rocha *et al*., 1999). The spacing between the rbs sequence and the AUG initiation codon ranges from 7 to 9 nucleotides, a distance that has been reported to be optimal for efficient translation (Vellanoweth and Rabinowitz, 1992). Moreover, analysis of the codon usage of *ptsH* and *ptsI* in *B. subtilis* show that both genes preferentially contain codons used in highly expressed genes and that this bias is more pronounced for *ptsH* (Reizer *et al*., 1993). These data suggest that the production of the very high levels of cellular HPr found in low G+C Gram-positive bacteria results from the combined effect of transcriptional and translational structural elements associated with optimal gene expression. On the other hand, there are fewer differences between the *ptsH* rbs and the *B. subtilis* consensus S-D sequence than between the *ptsI* rbs and the *B. subtilis* consensus S-D sequence (Figure 2B). Moreover, the distances between the *ptsI* rbs and the initation AUG codons range from 12 to 15 nucleotides. Considering the fact that the distance between the rbs and the AUG codon that promotes optimal translation is approximately 7 bases (Vellanoweth and Rabinowitz, 1992), translation of *ptsI* may be less efficient than that of *ptsH*. This may partially explain why cells contain lower levels of EI than HPr. Results obtained with a spontaneous mutant (mutant L26) from *S. salivarius* demonstrated that there is indeed a difference in efficiency between *ptsH* rbs and *ptsI* rbs (Gagnon *et al*., 1995). This mutant bears a 1 bp deletion (C) in codon 82 of *ptsH*, which generates a HPr-EI fusion protein. Translation of the fusion protein in L26 is initiated using the *ptsH* rbs, while translation of free EI is initiated using the *ptsI* rbs. Measurement of the HPr-EI fusion protein and free EI by Western blot has revealed that the cells synthesize six-fold more HPr-EI fusion protein than free EI, confirming the difference of efficiency between the *ptsH* rbs and the *ptsI* rbs. These results are consistent with the hypothesis that the intracellular HPr/EI ratio in low G+C Gram-positive bacteria is determined by both transcriptional and translational regulatory mechanisms.

## General Conclusion

The *pts* operon is composed of two genes, *ptsH* and *ptsI*, coding respectively for HPr and EI, the general energy coupling proteins of the PTS. Low G+C Gram-positive bacteria contain high amounts of HPr and EI. This observation is consistent with the finding that the *pts* promoter possesses all the features of the canonical promoter. Cells contain more HPr than EI, and the HPr/EI ratio varies among species and is influenced by growth conditions. Experimental results and *in silico* analyses suggest that the non co-ordinated synthesis of these two PTS proteins is controlled by transcriptional and translational mechanisms. Identification of the *trans* elements involved in these regulatory controls await future study. Recent results obtained with *pts* mutants of *S. salivarius* indicate that the rate of transcription as well as termination efficiency at the rho-dependent terminator located at the 5'-end of *ptsI* vary with cellular concentrations of HPr. This leads us to propose the heuristic hypothesis that the *pts* operon is subject to autogenous regulation by HPr.

#### Acknowledgements

We would like to thank M. Denis Brochu for making the illustrations and Gene Bourgeau for editing the manuscript. Research in the laboratories of CV and MF is supported by grants from the Medical Research Council of Canada (MOP-36338 and MT11276), the Fonds pour la Formation de Chercheurs et l'Aide à la Recherche (FCAR), and the Conseil des Recherches en Pêche et en Agroalimentaire du Québec (CORPAQ, grant 4486).

#### References

Ayers, D.G., Auble, D.T., and deHaseth, P.L. 1989. Promoter recognition by *Escherichia coli* RNA polymerase: role of the spacer DNA in functional complex formation. J. Mol. Biol. 207: 749-756.

Borst, D.W., and Bentley, M.J. 1994. Promoter analysis of the staphylococcal enterotoxin A gene. J. Biol. Chem. 269: 1883-1888.

Boyd, D.A., Cvitkovitch, D.G., and Hamilton, I.R. 1994. Sequence and expression of the genes for HPr (*ptsH*) and Enzyme I (*ptsI*) of the phosphoenolpyruvate-dependent phosphotransferase system from *Streptococcus mutans*. Infect. Immun. 62: 1156-1165.

Boyd, D.A., Cvitkovitch, D.G., and Hamilton, I.R. 1995. Sequence, expression, and function of the gene for the nonphosphorylating, NADP-dependent glyceraldehyde-3-phosphate dehydrogenase of *Streptococcus mutans*. J. Bacteriol. 177: 2622-2627.

Christensen, P.D., Benson, A.K., and Hutkins, W.R. 1998. Cloning and expression of the *Listeria monocytogenes* Scott A *ptsH* and *ptsI* genes,

coding for HPr and Enzyme I, respectively, of the phosphotransferase system. Appl. Environ. Microbiol. 64: 3147-3152.

Coburn, G.A., and Mackie, G.A. 1999. Degradation of mRNA in *Escherichia coli*: an old problem with some new twists. Prog. Nucleic Acid Res. Mol. Biol. 62: 55-108.

Cochu, A.C., Vadeboncoeur, C., and Frenette, M. 1999. Isolation and characterization of the *pts* operon from *Streptococcus thermophilus* ATCC 19258. ASM Annual meeting. Chicago, IL. USA. Abst. K88.

Eiserman, R., Fischer, R., Kessler, U., Neubauer, A. and Hengstenberg, W. 1991. Staphylococcal phosphoenolpyruvate-dependent phosphotransferase system. Purification and protein sequencing of the *Staphylococcus carnosus* histidine-containing protein, and cloning and DNA sequencing of the *ptsH* gene. Eur. J. Biochem. 197 : 9-14.

Fang, L., Hou, Y., and Inouye, M. 1998. Role of the cold-box region in the 5'-untranslated region of the *cspA* mRNA in its transient expression at low temperature in *Escherichia coli*. J. Bacteriol. 180: 90-95.

Gagnon, G., Vadeboncoeur, C., and Frenette, M. 1993. Phosphotransferase system of *Streptococcus salivarius* : characterization of the *ptsH* gene and its product. Gene 136 : 27-34

Gagnon, G., Vadeboncoeur, C., Gauthier, L., and Frenette, M. 1995. Regulation of the *ptsH* and *ptsI* gene expression in *Streptococcus salivarius* ATCC 25975. Mol. Microbiol. 16: 1111-1121.

Gagnon, G., Vadeboncoeur, C., Lévesque, R.C. and Frenette, M. 1992. Cloning, sequencing and expression in *Escherichia coli* of the *ptsI* gene encoding enzyme I of the phosphoenolpyruvate : sugar phosphotransferase transport system from *Streptococcus salivarius*. Gene 121 : 71-78.

Gauthier, L., Bourassa, S., Brochu, D., and Vadeboncoeur, C. 1990. Control of sugar utilization in oral streptococci. Properties of phenotypically distinct 2-deoxyglucose-resistant mutants of *Streptococcus salivarius*. Oral Microbiol. Immunol. 5: 352-359.

Gauthier, L., Thomas, S., Gagnon, G., Frenette, M., Trahan, L., and Vadeboncoeur, C. 1994. Positive selection for resistance to 2-deoxyglucose gives rise, in *Streptococcus salivarius*, to seven classes of pleiotropic mutants, including ptsH and ptsI missense mutants. Mol. Microbiol. 13: 1101-1109.

Gauthier, M., Brochu, D., Eltis, L.D., Thomas, S., and Vadeboncoeur, C. 1997. Replacement of isoleucine-47 by threonine in the HPr protein of *Streptococcus salivarius* abrogates the preferential metabolism of glucose and fructose over lactose and melibiose but does not prevent the phosphorylation of HPr on serine-46. Mol. Microbiol. 25: 695-705.

Gonzy-Treboul, G., Zagorek, M., Rain-Guion, M.-C., and Steinmetz, M. 1989. Phosphoenolpyruvate:sugar phosphotransferase system of *Bacillus subtilis*: nucleotide sequence of *ptsX*, *ptsH*, and the 5'-end of *ptsI* and evidence for a *ptsHI* operon. Mol. Microbiol. 3: 103-112.

Hamilton, I.R., and Buckley, N.D. 1991. Adaptation by *Streptococcus mutans* to acid tolerance. Oral Microbiol. Immunol. 6: 65-71.

Hamilton, I.R., Gauthier, L., Desjardins, B., and Vadeboncoeur, C. 1989. Concentration-dependent repression of the soluble and membrane components of the *Streptococcus mutans* phosphoenolpyruvate:sugar phosphotransferase system by glucose. J. Bacteriol. 171: 2942-2948.

Jenkinson, H.F. 1989. Properties of a phosphocarrier protein (HPr) extracted from intact cells of *Streptococcus sanguis*. J. Gen. Microbiol. 135: 3183-3197.

Kenney, T.J., and Churchward, G. 1996. Genetic analysis of the *Mycobacterium smegmatis rpsL* promoter sequence. J. Bacteriol. 164: 1353-1355.

Kolhlbrecher, D., Eisermann, R., and Hengstenberg, W. 1992. Staphylococcal phosphoenolpyruvate-dependent phosphotransferase system: molecular cloning and nucleotide sequence of the *Staphylococcus carnosus ptsI* gene and expression and complementation studies of the gene product. J. Bacteriol. 174: 2208-2214.

Kunst, F., Ogasawara, N., Moszer, I., Albertini, AM., Alloni, G., Azevedo, V., Bertero, MG., Bessieres, P., Bolotin, A., Borchert, S., Borriss, R., Boursier, L., Brans, A., Braun, M., Brignell, SC., Bron, S., Brouillet, S., Bruschi, CV., Caldwell, B., Capuano, V., Carter, NM., Choi, SK., Codani, JJ., Connerton, IF., Danchin, A., *et al.* 1997. The complete genome sequence of the gram-positive bacterium *Bacillus subtilis*. Nature, 390: 249-256.

Lai, X., and Ingram, L.O. 1995. Discovery of a *ptsHI* operon, which includes a third gene (*ptsT*), in the thermpophile *Bacillus stearothermophilus*. Microbiology, 141: 1443-1449.

Lortie, L.A., Gagnon, G., and Frenette, M. 1994. IS *1139* from *Streptococcus salivarius*: identification and characterization of an insertion sequence-like element related to mobile DNA elements from Gram-negative bacteria. Plasmid, 32: 1-9.

Luesink, E.J., Breumer, M.A., Kuipers, O.P., and de Vos, W.M. 1999. Molecular characterization of the *Lactococcus lactis ptsHI* operon and analysis of the regulatory rôle of HPr. J. Bacteriol. 181: 764-771.

Mattoo, R.L., and Waygood, E.B. 1983. Determination of the levels of HPr and enzyme I of the phosphoenolpyruvate-sugar phosphotransferase system in *Escherichia coli* and *Salmonella typhimurium*. Can. J. Biochem. Cell Biol. 61: 29-37.

Mitchell, W.J., Albasheri, K.A., and Yazdanian, M. 1995. Factors affecting utilization of carbohydrates by clostridia. FEMS Microbiol. Rev. 17: 317-329.

Miwa, Y., Nakata, A., Ogiwara, A., Yamamoto, M., and Fujita, Y. 2000. Evaluation and characterization of catabolite-responsive elements (cre) of *Bacillus subtilis*. Nucleic Acids Res. 28: 1206-1210.

Parker, C., and Hutkins, R.W. 1997. *Listeria monocytogenes* Scott A transports glucose by high-affinity and low-affinity glucose transport systems. Appl. Environ. Microbiol. 63: 543-546.

Plamondon, P., Brochu, D., Thomas, S., Fradette, J., Gauthier, L., Vaillancourt, K., Buckley, N., Frenette, M., and Vadeboncoeur, C. 1999. Phenotypic consequences resulting from a methionine-to-valine substitution at position 48 in the HPr protein of *Streptococcus salivarius*. J. Bacteriol. 181: 6914-6921.

Postma, P.W., Lengeler, J.W., and Jacobson, G.R. 1993. Phosphoenolpyruvate:carbohydrate phosphotransferase systems of bacteria. Microbiol. Rev.57: 543-594.

Reizer, J., Hoischen, C., Reizer, A., Pham, T.N., and Saier, M.H.Jr. 1993. Sequence analysis and evolutionary relationships among the energy-coupling proteins Enzyme I and HPr of the bacterial phosphoenolpyruvate:sugar phosphotransferase system. Protein Science, 2: 506-521.

Reizer, J., Novotny, M.J., Hengstenberg, W., and Saier, M.H.Jr. 1984. Properties of ATP-dependent protein kinase from *Streptococcus pyogenes* that phosphorylates a seryl residue in HPr, a phosphocarrier protein of the phosphotransferase system. J. Bacteriol. 160: 333-340.

Reizer, J., Sutrina, S. L., Wu, L.F., Deutscher, J., Reddy, P., and Saier, M.H.Jr. 1992. Functional interactions between proteins of the phosphoenolpyruvate:sugar phosphotransferase systems of Bacillus subtilis and *Escherichia coli*. J. Biol. Chem. 267: 9158-9169.

Rocha, E.P.C., Danchin, A., and Viari, A. 1999. Translation analysis in *Bacillus subtilis*: roles and trends on initiation and termination, insights from a genome analysis. Nucleic Acids Res. 27: 3567:3576.

Rodrigue, L., Lacoste, L., Trahan, L., and Vadeboncoeur, C. 1988. Effect of nutritional constraints on the biosynthesis of the components of the phosphoenolpyruvate:sugar phosphotransferase system in a fresh isolate of *Streptococcus mutans*. Infect. Immun. 56: 518-522.

Romano, A.H., Eberhard, S.J., Dingle, S.L., and McDowell, T.D. 1970. Distribution of the phosphoenolpyruvate:glucose phosphotransferase system in bacteria. J. Bacteriol. 104: 808-813.

Romano, A.H., Trifone, J.D., and Brustolon, M. 1979. Distribution of the phosphoenolpyruvate:glucose phosphotransferase system in fermentative bacteria. J. Bacteriol. 139: 93-97.

Saier, M.H.Jr., Chauvaux, S., Cook, G.M., Deutscher, J., Paulsen, I.T., Reizer, J., and Ye, J.J. 1996. Catabolite repression and inducer control in Gram-positive bacteria. Microbiology, 142: 217-230.

Saier, M.H.Jr., and Reizer, J. 1992. Proposed uniform nomenclature for the proteins and protein domains of the bacterial phosphoenolpyruvate:sugar phosphotransferase system. J. Bacteriol. 174: 1433-1438.

Saier, M.H.Jr., and Reizer, J. 1994. The bacterial phosphotransferase system: new frontiers 30 years later. Mol. Microbiol. 13: 755-764.

Stentz, R., Lauret, R., Ehrilch, S.D., Mortel-Deville, F., and Zagorek, M. 1997. Molecular cloning and analysis of the *ptsHI* operon in *Lactobacillus sake*. Appl. Environ. Microbiol. 63: 2111-2116.

Stülke, J., Martin-Verstraete, I., Zagorek, M., Rose, M., Klier, A., and Rapoport, G. 1997. Induction of the *Bacillus subtilis ptsGHI* operon by glucose is controlled by a novel antiterminator, GlcT. Mol. Microbiol. 25: 65-78.

Stülke, J., Arnaud, M., Rapoport, G., and Martin-Verstraete, I. 1998. PRD-a protein domain involved in PTS-dependent induction and carbon catabolite repression of catabolic operons in bacteria. Mol. Microbiol. 28: 865-874.

Thevenot, T., Brochu, D., Vadeboncoeur, C., and Hamilton, I.R. 1995. Regulation of ATP-dependent P-(Ser)-HPr formation in *Streptococcus mutans* and *Streptococcus salivarius*. J. Bacteriol. 177: 2751-2759.

Thibault, L., and Vadeboncoeur, C. 1985. Phosphoenolpyruvate-sugar phosphotransferase transport system of Streptococcus mutans: purification of HPr and Enzyme I and determination of their intracellular concentrations by rocket immunoelectrophoresis. Infect. Immun. 50: 817-825.

Thomas, S., Roy, A., Frenette, M., and Vadeboncoeur, C. 1997. Effects of point mutations in the promoter region of the *pts* operon of *Streptococcus salivarius*. Meeting of the American Society for Microbiology, Miami, USA. Abstract K84.

Vadeboncoeur, C., Brochu, D., and Reizer, J. 1991. Quantitative determination of the intracellular concentration of the various forms of

HPr, a phosphocarrier protein of the phosphoenolpyruvate:sugar phosphotransferase system in growing cells of oral streptococci. Anal. Biochem. 196: 24-30.

Vadeboncoeur, C., Proulx, M., and Trahan, L. 1983. Purification of proteins similar to HPr and enzyme I from the oral bacterium *Streptococcus salivarius.* Biochemical and immunochemical properties. Can. J. Microbiol. 29: 1694-1705.

Vadeboncoeur, C., Thibault, L., Néron, S., Halvorson, H., and Hamilton, I.R. 1987. Effect of growth conditions on levels of components of the phosphoenolpyruvate:sugar phosphotransferase system in *Streptococcus mutans* and *Streptococcus sobrinus* grown in continuous culture. J. Bacteriol. 169, 5686-5691.

Vellanoweth, R.L., and Rabinowitz, J.C. 1992. The influence of ribosome-binding site elements on translational efficiency in *Bacillus subtilis* and *Escherichia coli in vivo*. Mol. Microbiol. 6: 1105-1114.

Viana, R., Monedero, V., Dossonet, V., Vadeboncoeur, C., Pérez-Martinez, G., and Deutscher, J. 2000. Enzyme I and HPr from *Lactobacillus casei*: their role in sugar transport, carbon catabolite repression and inducer exclusion. Mol. Microbiol. 36: 570-584.

Voskuil, M.I., and Chambliss, G.H. 1998. The –16 region of *Bacillus subtilis* and other Gram-positive bacterial promoters. Nucleic Acids Res. 26: 3584-3590.

Voskuil, M.I., Voepel, K., and Chambliss, G.H. 1995. The –16 region, a vital sequence for utilization of a promoter in *Bacillus subtilis* and *Escherichia coli*. Mol. Microbiol. 17: 271-279.