J. Life Science. (2001), 11(2), 120~125

JOURNAL OF
Life Science

Regulation of Expression of the *Bacillus caldolyticus* Pyrimidine Biosynthetic Operon by *pyrR* Gene, an Autogenous Regulator

Sa-Youl Ghim†

Department of Microbiology, College of Natural Sciences, Kyungpook National University, #1370 Sankyug-Dong, Buk-Gu, Daegu, 702-701 Korea

Received: August 11, 2001

Abstract The *pyrR* gene of the pyrimidine biosynthesis (*pyr*) operon of the thermophile *Bacillus caldolyticus*, encoding a uracil phosphoribosyltransferase (UPRTase), turned to role as a *pyr* operon regulator. It has been proposed that PyrR mediates transcriptional termination-antitermination at three intercistronic regions of the *pyr* operon (S.-Y. Ghim and J. Neuhard, *J. Bacteriol.*, **176**, 3698-3707, 1994). In this research, a plasmid carrying the *pyrR* region of *B. caldolyticus* could restore a pyrimidine regulation in a *pyrR* mutant of *B. subtilis*. Expression of *pyrR* was found to increase 6-7 fold during pyrimidine starvation. Additionally, a highly conserved nucleotide sequence which may constitute the binding site for a PyrR protein (PyrR-binding loop) in transcript was suggested. Alternative antiterminator and terminator structures involving three conserved motifs in front of the *pyrR, pyrP* and *pyrB* genes, respectively, are proposed to account for the observed regulation pattern.

Key words: *pyr* operon, *pyrR*, pyrimidine-mediated regulation, *B. caldolyticus*.

Introduction

Pyrimidine nucleotide synthesis and its control has extensively been studied in enteric bacteria and bacilli [1,2]. The genes encoding pyrimidine synthesis enzymes of *Escherichia coli* and *Salmonella typhimurium* are scattered around the chromosome. In contrast, the pyrimidine nucleotide biosynthetic (*pyr*) genes in *Bacillus subtilis*, *B. caldolyticus*, and *B. halodurans* have been found to be closely linked in a cluster on the chromosome, in the order promoter-*pyrR-PyrP-pyrB-pyrC-pyrAA-pyrAB-pyrDII-pyrDI-pyrF-pyrE* [3-6].

It has been proposed and partially proved that the product of the first open reading frame, *pyrR*, functions as the *pyr* regulatory protein and also possesses uracil phosphoribisyltransferase (UPRTase) activity, in bacilli [3,6]. In *B. subtilis*, it has been completely shown that pyrimidine *de novo* biosynthetic operon is controlled by a regulatory protein PyrR-mediated attenuation [7,8]. Exogenous pyrimidines act to promote termination at three attenuation sites located in the 5 leader, *pyrR-pyrP*, and *pyrP-pyrB* intergenic regions. Each attenuation site on *pyr* mRNA could be folded into three secondary structures to function as a transcriptional terminator, an antiterminator, and an anti-antiterminator (binding loop for PyrR protein).

Independently, the exactly same mechanism has been proposed for expression of the *pyr* operon of *B. caldolyticus* [3], since the very similar arrangement of genes and regulatory sites have been found in the *B. caldolyticus pyr* operon. Further characterization of the *B. caldolyticus pyr* regulation has not done at all, however. Here experimental evidences that the *pyrR* gene of *B. caldolyticus* encodes a regulatory protein that affects *pyr* expression are presented.

Materials and Methods

Bacterial strains, plasmids, and growth conditions

The bacterial strains and plasmids used in this work are listed in Table 1. *B. caldolyticus* was grown aerobically at 60℃ in mineral salts medium 162 [12]. *B. subtilis* was grown at 37℃ in Spizizen salt minimal medium [13] or CH minimal medium [14] for selection of *pyrR* mutants and when making competent cells, in GM medium [15]. *E. coli* was grown at 37℃ in AB minimal medium [16]. LB medium [17] was used as a rich medium for growth of all these strains, except that 0.5% glucose and 5 mM $CaCl_2$ were added for growth of *B. caldolyticus*.

Isolation of *pyrR* mutants in *B. subtilis*

B. subtilis pyrR mutants were isolated from strain JH861 (*trpC2, cpa*) which lacks the arginine-repressible carbamyl phosphate synthetase, but is able to grow on minimal medium without arginine, since carbamyl phosphate is supplied by the pyrimidine-repressible carbamyl phosphate synthe-

†Corresponding author
Phone: 82-53-950-5374, Fax: 053-955-5522
E-mail: ghimsa@knu.ac.kr

Table 1. Bacterial strains and plasmids

Strain or plasmid	Relevant characteristics	Reference or source
Bacillus caldolyticus		
DSM405	wild type	DSM[a]
Bacillus subtilis		
JH861	*trpC2*, sensitive to uracil	[9]
BKU105	*trpC2*, *pyrR*, resistant to uracil and uridine from JH861	This study
DB104	*his*, *nprR2*, *nprE18*, *aprA3*	[10]
DB104 Δ*pyrR*	HC11, *pyrB*$^+$, spec^S, *pyrR*	
ED364	*pyrA*	BGSC[b] 1A391
ED383	*trpC2*, UPRT[c]	[3]
Plasmids		
pHPS9	Cm^R, Er^R, *Cat-86::lacZ* α	[11]
pHPSY1	2.6-kb *Eco*RI-HindIII fragment with *pyrR* and *pyrP* in the *Eco*RI-*Hin*dIII sites of pHPS9	[3]
pHPSY2	1.2-kb *Eco*RI-*Sma*I fragment with *pyrR* in the *Eco*RI-*Sma*I sites of pHPS9	[3]
pHPSY3	pHPSY1 with deleted *Bst*EII overhang in *pyrR*	[3]

[a]DSM, Deutsche Sammlung für Mikroorganismen und Zell Kulturen, Braunschweig, Germany.
[b]BGSC, *Bacillus subtilis* Genetic Stock Center, Ohio State University, Columbus, Ohio.
[c]UPRT$^-$, Lacking uracil phosphoribosyltransferase activity.

tase encoded by *pyrAA* and *pyrAB* genes [18].

Spontaneous pyrimidine-tolerant revertants of strain JH861 were selected by the procedure described by Ghim and Switzer [8]. The pyrimidine-tolerant mutants were screened by plating 1 ml of an overnight culture in LB medium on CH minimal medium plates containing 50 μg ml^{-1} each of uracil and uridine.

General DNA techniques

DNA and RNA techniques were applied according to standard [19] and suppliers' protocols. Development of competence and transformation in *B. subtilis* were done as described by Boylan *et al.* [15] and for *E. coli* as described by Cohen *et al.* [20]. DNA was sequenced by the dideoxy chain-termination method [21].

PCR amplification

PCR amplification of genomic DNA of the *B. subtilis pyrR* mutants was performed with an GeneAmp® PCR System 2700 (Applied Biosystems) as described by the manufacturer. PCR primers used for amplifying a 860-bp chromosomal DNA fragment that included the whole *pyrR* gene were PyrR-A1 (5'-CGCGTTCCCGAGGATATGGC-3') and PyrR/Rev (5'-GACCTGCCGAATACTTTTTGG-3') as described previously [8].

Each 100-$\mu\ell$ reaction mixture contained 50 mM KCl, 10 mM Tris-HCl (pH 8.4), 1.5 mM $MgCl_2$, 50 μM each deoxynucleoside triphosphate, 0.5 pmol of each primer, 1 μg of template DNA per ml, and 2 U of AmpliTaq DNA polymerase [7]. The reaction mixture was heated to 94℃ for 3 min and passed through 30 reaction cycles as follows: denaturation at 94℃ for 1 min, primer annealing at 55℃ for 30 s, and extension at 72℃ for 45 s. After the completion of the cycles, the reaction was extended at 72℃ for 15 min.

Enzyme assays

An exponentially growing culture with an OD_{436} of about 1.0 (3×10^8 cells/ml) was harvested by centrifugation, washed in 0.9% NaCl, and resuspended in the appropriate buffer for each enzyme assay. The cell suspension was sonicated at 4℃ for 45 sec and, when desired, centrifuged at 5,000 rpm for 10 min. Either the sonicated extract or the supernatant was used for enzyme assays.

Uracil phosphoribosyltransferase (UPRTase) was assayed by measuring the PRPP-dependent conversion of [^{14}C]uracil to [^{14}C]UMP [22]. Aspartate transcarbamylase (ATCase) activity was determined by the method of Prescott and Jones [23]. Protein content was determined by using the Coomassie protein assay reagent (Pierce, Rockford, Ill.) with bovine serum albumin as the standard.

Computer analysis

RNA secondary structure was predicted by the FOLD program [39]. Computer analyses of nucleotide and amino acid sequences were carried out with DNA Strider and DNA Star programs.

Results and Discussion

Isolation and characterization of a *pyrR* mutant

BKU105, a spontaneous pyrimidine-tolerant revertant of *B. subtilis* strain JH861 which is initially uracil-sensitive, was selected by the procedure described previously [8]. The pyrimidine-tolerant mutants were found at a frequency of about $4\text{-}5\times10^{-8}$.

Specific activity of ATCase (nmol · mg^{-1}min^{-1}) for the

BKU105 mutant strain grown in minimal medium without any pyrimidine sources, was 4769, which is much higher level compared to 464 of the parent strain JH861. With uracil and uridine (50 μg ml^{-1} each), the BKU105 strain showed specific activity of 4620. These data demonstrated that the BKU105 mutant strain was strongly derepressed in *pyrB* expression and was resistant to repression by exogenous pyrimidines.

Sequencing the DNA fragment encoding PyrR from the BKU105 showed that translational termination mutation occurred at a codon near the N-terminus of PyrR (data not shown).

Complementation of *pyrR* mutants

It was investigated whether the presence of the *B. caldolyticus pyrR* gene could restore the regulation control by pyrimidine in *B. subtilis pyrR* mutants. For *pyrR* complementation, plasmid constructs pHPSY1, pHPSY2, and pHPSY3 (Fig. 1) were transformed in *B. subtilis* JH861 and BKU105 and tested 20 μg/ml uracil sensitivity. As shown in Fig. 2, either plasmid pHPSY1 or pHPSY2 which both contain the *B. caldolyticus pyrR* could restore uracil sensitivity of *B. subtilis* BKU105, whereas pHPSY3 which has a frame-shift *Bst*EII deletion in the *pyrR*, showed uracil resistance. In the *B. subtilis pyrR* mutants DB104 *⊿pyrR* which shows no regulation with or without uracil and high expression level of aspartate transcarbamylase encoded by *pyrB*, the presence of either plasmid pHPSY1 or pHPSY2 recovered the availability of pyrimidine (Table 2), whereas the presence of pHPSY3 did not change at all. These results suggested that the *pyrR* gene is responsible for regulatory function in pyrimidine biosynthesis.

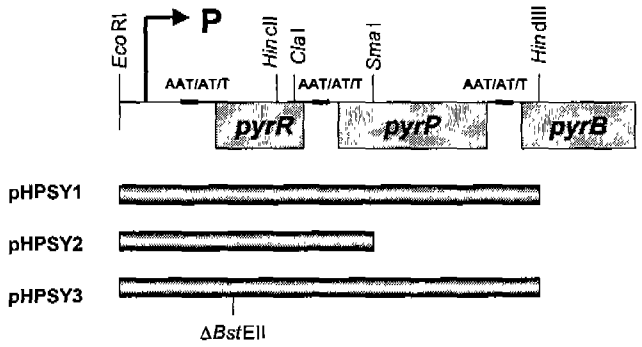

Fig. 1. Structure of the 5 end of the *pyr* region from *B. caldolyticus*. Open boxes indicate the ORFs of individual *pyr* genes. The bent arrow with P (promoter) represents the transcriptional start of the *pyr* operon. Locations of proposed anti-antiterminator (AAT, binding loop)/antiterminator (AT)/terminator (T) structures on transcripts are indicated by AAT/AT/T. Filled bars indicate fragments obtained by subcloning in pHPS9 vector as described in Table 1. *⊿BstEII* means the removal of *Bst*EII site by treatment of mung bean nuclease followed by blunt-end ligation. Fig adapted with minor changes from Ghim and Neuhard [3].

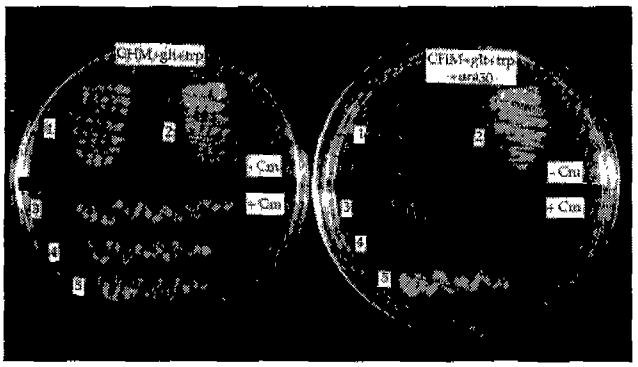

Fig. 2. Complementation test of the *B. caldolyticus pyrR* in *B. subtilispyr* regulatory mutants. The *B. caldolyticus pyrR* complements *B. subtilis pyr* regulatory mutation. Growth of JH861 (1) and BKU105 (2) containing pHPSY1 (3), pHPSY2 (4), and pHPSY3 (5) respectively on only Cox & Hanson minimal medium plate (left) [14], or with 50 μg/ml uracil (right), with (both lower) or without (both upper) 5 μg/ml chloramphenicol (Cm).

Table 2. Expression of multicopy plasmids carrying *B. caldolyticus pyrR* inserts in *B. subtilis pyrR* knock-out mutant

Strain	Plasmid	Specific activities of ATCase (nmole/mg · min)		
		-uracil	+uracil	-/+[a]
DB104		370	28	13.2
DB104 *⊿*pyrR		3,055	3,364	0.9
DB104 *⊿*pyrR	pHPSY1	545	62	8.8
DB104 *⊿*pyrR	pHPSY2	483	56	8.6
DB104 *⊿*pyrR	pHPSY3	3,155	3,242	1.0

[a]-/+, fold repression (-uracil activity/+uracil activity).

More interesting than the great similarities between the *pyr* systems from Gram-positive organisms are their differences [25]. The *pyr* operons of the various organisms have now been described that are regulated by PyrR-dependent attenuation at one (*Enterococcus faecalis*) [25], two (*Lactobacillus plantarum*) [26] or three (*B. subtilis* and *B. caldolyticus* clustered and *Lactococcus lactis* scattered on each chromosome) [3,27,6] sites in their operons. It is intriguing to consider that all three arrangements presumably provide adequate regulation of the pyrimidine biosynthetic enzymes for their host organisms.

Pyrimidine regulation of *pyrR* expression

In general, it has been known that expression of the genes involved in pyrimidine nucleotide biosynthesis is regulated by the pyrimidine supply. In the present work, it was found that expression of the *B. caldolyticus pyrR* gene in *B. subtilis* is not repressed by the addition of uracil under the conditions used. It could be explained by the facts that *B. subtilis* chromosome includes two genes, *upp* and *pyrR*, encoding UPRTase and the major products of UPRTase in the cells are encoded by the former *upp* gene [28]. As shown

in Table 3, the presence of uracil in the growth medium had no effect on the level of UPRTase in wild type *B. caldolyticus* grown at 60℃; nor did it affect expression of *B. caldolyticus* UPRTase from the plasmid pHPSY2 when present in *B. subtilis* ED383. Much less UPRTase activity in plasmid-harbouring strains than in wild-type strains might mean the followings: 1) the *pyrR* gene could encode very tiny amounts of UPRTase even amplified by vector plasmid; and 2) the host ED383 strain lost whole UPRTase from both *pyrR* and *upp* genes.

However, pyrimidine starvation induced in the *B. subtilis pyrA* mutant ED364 by addition of arginine resulted in a 6-7 fold increase in the level of UPRTase expressed from pHPSY2. The rationale for using ED364 was the pyrimidine specific carbamylphosphate synthetase encoded by the *car* operon. Thus, addition of arginine causes pyrimidine starvation [29].

Transcription signals

When the three spacer regions between the promoter and the *pyrR* gene and among the *pyrR*, *pyrP* and *pyrB* genes were analyzed for possible secondary structures, it was found that each space contains two regions of partial dyad symmetry [3]. The RNA in these intergenic regions may form one of two competing secondary structures. The smaller one resembles a Rho-independent transcriptional terminator (T) consisting of GC rich stem-loop structure, followed by a stretch of uridine residues. The larger structures are overlapped with most of the left stem of the putative terminator structures, which would prevent the formation of the terminator hairpin. These rather big secondary structures can thus serve as antiterminators (AT).

The quite different regulation of the expression of the *B. caldolyticus pyrR* and *pyrB* genes under the conditions of uracil addition suggested that the presence of one (for *pyrR*), two (for *pyrP*). or three (for all *pyr* genes) AT-T structures may lead to differential regulation. Transcriptional termination-antitermination control mechanisms, mediated by RNA-binding proteins, have been shown to occur for several genes and operons in *B. subtilis* including the following: *bgl* [30], *trp* [31,32], *sacB* [33], *pur* [34], *sacPA* [35], and *glpD* [36]. The most well characterized system is the *trp* operon [37].

In addition, a highly conservative region of about 60-bp was found in each T-AT module in *B. caldolyticus pyr* operon. As shown in Fig. 3, these putative regulatory motifs overlap the AT regions but not the T regions. This conserved sequence could be the binding site for an RNA binding protein and it could be envisioned that binding of the protein to the binding site would preclude the formation of the AT structure, leading to transcription termination, as illustrated in Fig. 4. Under high UMP conditions, the hypothetical RNA binding protein would be in its active conformation, the T structure will form and transcription will be terminated. Under low UMP conditions, the binding protein is inactive, the AT structure will form and lead to transcriptional readthrough. It should be noted, however, that no direct evidence for this mechanism has yet been obtained in *B. caldolyticus pyrR* control system.

In contrast, it has clearly shown that purified PyrR protein could bind with high affinity and specificity to the putative conserved binding motifs on mRNA in *B. subtilis* and *E. faecalis*, by gel mobility shift analysis [25,38]. Moreover, the crystal structure of *B. subtilis* PyrR protein has been determined and the structure is consistent with the idea that PyrR regulatory function is independent of catalytic activity [39]. Since the *B. caldolyticus* UPRTase is very thermostable, its crystal would solve the previous problem that the unstable *B. subtilis* PyrR crystal hardly got binding to the precise target binding motifs on mRNA, which is under studying [collaboration with R. L Switzer].

Table 3. Effect of pyrimidine starvation on UPRTase activity[a]

Strain	Plasmid	Control compounds (μg/ml)	UPRTase Specific activity[b]	Assay temp
B. caldolyticus		-	12.4	60℃
DSM405		uracil (20)	11.5	60℃
B. subtilis		-	13.6	37℃
168		uracil (20)	13.3	37℃
B. subtilis	pHPSY2	-	1.5	60℃
ED383		uracil (20)	1.6	60℃
B. subtilis	pHPSY2	-	3.8	60℃
ED364		arginine (50)/ uracil (20)	1.2	60℃
		arginine (50)	8.0	60℃

[a]Cells were grown exponentially in minimal medium with additions as indicated.
[b]nmole × min^{-1} × mg^{-1} protein.

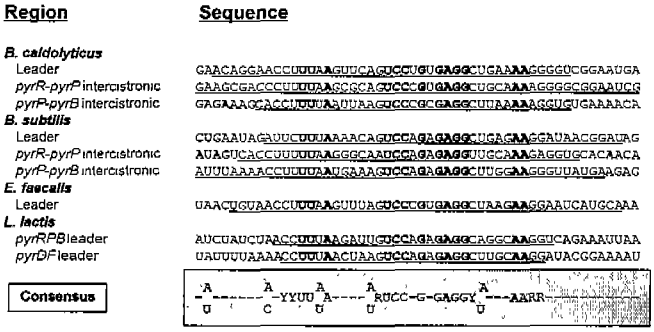

Fig. 3. Identification of a conserved putative PyrR binding sequence in the three anti-antiterminator regions of the *B. caldolyticus pyr* operon transcript. The sequences thought to form an anti-antiterminator stem-loop in each sequence are underlined, and nucleotides that are identical in all of the sequences are bolded. The resulting consensus sequence is shown at the bottom box. The sequences are from *B. caldolyticus*, *B. subtilis*, *E. faecalis*, and *L. lactis* [3,25,27,6].

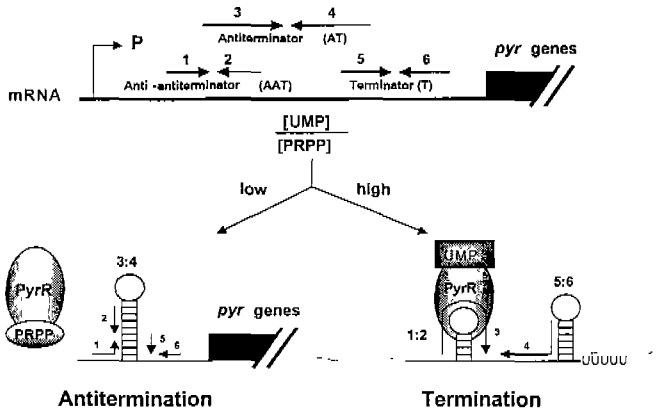

Fig. 4. Transcriptional termination-antitermination control mode of *pyr* gene expression in *B. caldolyticus*. The arrow with P indicates the promoter and the transcriptional start. A highly conserved sequence for PyrR protein is indicated by segments 1 and 2 called anti-antiterminator (AAT) structure or PyrR binding loop. Under low UMP conditions, antiterminator (AT, segments 3 and 4) structure forms (3 : 4), which leads transcription continued. Under high UMP conditions, the PyrR protein activated with UMP compound binds to the AAT structure (1 : 2), which allows terminator structure (T, segments 5 and 6) to form (5 : 6).

Acknowledgements

I thank Jan Neuhard and Robert L. Switzer for their insights and encouragement during this project and Robert J. Turner for providing the *B. subtilis pyrR* knock-out mutant DB104 *ΔpyrR*.

This research was supported by Kyungpook National University Research Fund, 2000.

References

1. Neuhard, J. and P. Nygaard. 1987. Purines and pyrimidines, pp. 445-473, *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter and H. E. Umbarger (eds.), *Escherichia coli and Salmonella typhimurium: cellular and molecular biology*. American Society for Microbiology, Washington, D.C.
2. Switzer, R. L. and C. L. Quinn. 1993. *De novo* pyrimidine nucleotide synthesis, pp. 343-358, *In* Sonenshein, A. L., J. A. Hoch, and R. Losick (eds.), *Bacillus subtilis and other Gram-positive bacteria: biochemistry, physiology, and molecular genetics*. American Society for Microbiology, Washington, D.C.
3. Ghim, S.-Y. and J. Neuhard. 1994. The pyrimidine biosynthesis operon of the thermophile *Bacillus caldolyticus* includes genes for uracil phosphoribosyltransferase and uracil permease. *J. Bacteriol.* **176**, 3698-3707.
4. Ghim, S.-Y., P. Nielsen and J. Neuhard. 1994. Molecular characterization of pyrimidine biosynthesis genes from the thermophile *Bacillus caldolyticus*. *Microbiology* **140**, 479-491.
5. Takami, H., K. Nakasone, Y. Takaki, G. Maeno, R. Sasaki, N. Masui, F. Fuji, C. Hirama, Y. Nakamura, N. Ogasawara, S. Kuhara and K. Horikoshi. 2000. Complete genome sequence of the alkaliphilic bacterium *Bacillus halodurans* and genomic sequence comparison with *Bacillus subtilis*. *Nucleic Acids Res.* **28**, 4317-4331.
6. Turner, R. J., Y. Lu and R. L. Switzer. 1994. Regulation of the *Bacillus subtilis* pyrimidine biosynthetic (*pyr*) gene cluster by an autogenous transcriptional attenuation mechanism. *J. Bacteriol.* **176**, 3708-3722.
7. Ghim, S.-Y. and R. L. Switzer. 1996. Characterization of *cis*-acting mutations in the first attenuator region of the *Bacillus subtilis pyr* operon that are defective in pyrimidine-mediated regulation of expression. *J. Bacteriol.* **178**, 2351-2355.
8. Ghim, S.-Y. and R. L. Switzer. 1996. Mutations in *Bacillus subtilis* PyrR, the *pyr* regulatory protein, with defects in regulation by pyrimidines. *FEMS Microbiol. Lett.* **137**, 13-18.
9. Paulus, T. J. and R. L. Switzer. 1979. Characterization of pyrimidine-repressible and arginine-repressible carbamyl phosphate synthetase from *Bacillus subtilis*. *J. Bacteriol.* **137**, 82-91.
10. Kawamura, F. and R. H. Doi. 1984. Construction of *Bacillus subtilis* double mutant deficient in extracellular alkaline and neutral proteases. *J. Bacteriol.* **160**, 442-444.
11. Haima, P., D. Van Sinderen, H. Schotting, S. Bron and G. Venema. 1990. Development of a β-galactosidase α-complementation for molecular cloning in *Bacillus subtilis*. *Gene* **86**, 63-69.
12. Degryse, E., N. Glansdorff and A. Piérard. 1978. A comparative analysis of extreme thermophilic bacteria belonging to the genus *Thermus*. *Arch. Microbiol.* **117**, 189-196.
13. Spizizen, J. 1958. Transformation of biochemically deficient strains of *Bacillus subtilis* by deoxyribonucleate. *Proc. Natl. Acad. Sci. USA* **44**, 1072-1078.
14. Cox, D. P. and R. S. Hanson. 1968. Catabolite repression of aconitate hydratase in *Bacillus subtilis*. *Biochim. Biophys. Acta* **158**, 36-44.
15. Boylan, R. J., N. H. Mendelson, D. Brooks and F. E. Young. 1972. Regulation of the bacterial cell wall: analysis of a mutant of *Bacillus subtilis* defective in biosynthesis of teichoic acid. *J. Bacteriol.* **110**, 281-290.
16. Clark, D. J. and O. Maaløe. 1967. DNA replication and the cell division cycle in *Escherichia coli*. *J. Mol. Biol.* **23**, 99-112.
17. Bertani, G. 1951. Studies of lysogenesis. I. The mode of phage liberation by lysogenic *Escherichia coil*. *J. Bacteriol.* **62**, 293-300.
18. Quinn, C. L., B. T. Stephenson and R. L. Switzer. 1991. Functional organization and nucleotide sequence of the *Bacillus subtilis* pyrimidine biosynthetic operon. *J. Biol. Chem.* **266**, 9113-9127.
19. Sambrook, J. and D. W. Russel. 2001. Molecular cloning: a laboratory manual, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
20. Cohen, S. N., A. C. Y. Chang and L. Hsu. 1972. Nonchromosomal antibiotic resistance in bacteria: genetic transformation of *Escherichia coli* by R-factor DNA. *Proc. Natl. Acad. Sci. USA* **69**, 2110-2114.
21. Sanger, F., S. Nicklen and A. R. Coulson. 1977. DNA sequencing with chain terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**, 5463-5467.
22. Rasmussen, U. B., B. Mygind and P. Nygaard. 1986. Purification and some properties of uracil phosphoribosyltransferase from *Escherichia coli* K12. *Biochim. Biophys. Acta* **881**, 268-275.

23. Prescott, L. M. and M. E. Jones. 1969. Modified methods for the determination of carbamyl aspartate. *Anal. Biochem.* **32**, 408-419.
24. Zuker, M. and P. Stiegler. 1981. Optimal computer folding of large RNA sequences using thermodynamics and auxiliary information. *Nucleic Acids Res.* **9**, 133-148.
25. Ghim, S.-Y., C. C. Kim, E. R. Bonner, J. N. D'elia, G. K. Grabner and R. L. Switzer. 1999. The *Enterococcus faecalis pyr* operon is regulated by autogenous transcriptional attenuation at a single site in the 5' leader. *J. Bacteriol.* **181**, 1324-1329.
26. Elagoz, A., A. Abdi, J. C. Hubert and B. Kammerer. 1996. Structure and organisms of the pyrimidine biosynthesis pathway genes in *Lactobacillus plantarum*: a PCR strategy for sequencing without cloning. Gene **181**, 37-43.
27. Martinussen, J., J. Schallert, B. Andersen and K. Hammer. 2001. The pyrimidine operon *pyrRPB-carA* from *Lactococcus lactis*. *J. Bacteriol.* **183**, 2785-2794.
28. Martinussen, J., P. Glaser, P. S. Andersen and H. H. Saxild. 1995. Two genes encoding uracil phosphoribosyltransferase are present in *Bacillus subtilis*. *J. Bacteriol.* **177**, 271-274.
29. Paulus, T. J., T. J. McGarry, P. G. Shekelle, S. Rosenzweig and R. L. Switzer. 1982. Coordinate synthesis of the enzymes of pyrimidine biosynthesis in *Bacillus subtilis*. *J. Bacteriol.* **149**, 775-778.
30. Murphy, N., D. J. McConnell and B. A. Cantwell. 1984. The DNA sequence of the gene and genetic control sites for the excreted *B. subtilis* enzyme β-glucanase. *Nucleic Acids Res.* **12**, 5355-5367.
31. Kuroda, M. I., D. Henner and C. Yanofsky. 1988. *cis*-Acting sites in the transcript of the *Bacillus subtilis trp* operon regulate expression of the operon. *J. Bacteriol.* **170**, 3080-3088.
32. Shimotsu, H., M. I. Kuroda, C. Yanofsky and D. J. Henner. 1986. Novel form of transcription attenuation regulates expression of the *Bacillus subtilis* tryptophan operon. *J. Bacteriol.* **166**, 461-471.
33. Shimotsu, H. and D. J. Henner. 1986. Modulation of *Bacillus subtilis* levansucrase gene expression by sucrose and regulation of the steady-state mRNA level by *sacU* and *sacQ* genes. *J. Bacteriol.* **168**, 380-388.
34. Shin, B. S., A. Stein and H. Zalkin. 1997. Interaction of *Bacillus subtilis* purine repressor with DNA. *J. Bacteriol.* **179**, 7394-7402.
35. Debarbouille, M., M. Arnaud, A. Fouet, A. Klier and G. Rapoport. 1990. The *sacT* gene regulating the *sacPA* operon in *Bacillus subtilis* shares strong homology with transcriptional antiterminators. *J. Bacteriol.* **172**, 3966-3973.
36. Holmberg, C. and B. Rutberg. 1991. Expression of the gene encoding glycerol-3-phosphate dehydrogenase (*glpD*) in *Bacillus subtilis* in controlled by antitermination. *Mol. Microbiol.* **5**, 2891-2900.
37. Antson, A. A., J. Otridge, A. M. Brzozowski, E. J. Dodson, G. G. Dodson, K. S. Wilson, T. M. Smith, M. Yang, T. Kurecki and P. Gollnick. 1995. The structure of *trp* RNA-binding attenuation protein. *Nature* **374**, 693-700.
38. Turner, R. J., E. R. Bonner, G. K. Grabner and R. L. Switzer. 1998. Purification and characterization of *Bacillus subtilis* PyrR, a bifunctional *pyr* mRNA-binding attenuation protein/uracil phosphoribosyltransferase. *J. Biol. Chem.* **273**, 5932-5938.
39. Tomchick, D. R., R. J. Turner, R. L. Switzer and J. L. Smith. 1998. Adaptation of an enzyme to regulatory function: structure of *Bacillus subtilis* PyrR, a pyr RNA-binding attenuation protein and uracil phosphoribosyltransferase. *Structure* **6**, 337-350.