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dra-nupC-pdp Operon of *Bacillus subtilis*: Nucleotide Sequence, Induction by Deoxyribonucleosides, and Transcriptional Regulation by the *deoR*-Encoded DeoR Repressor Protein

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The genes encoding deoxyriboaldolase (*dra*), nucleoside uptake protein (*nupC*), and pyrimidine nucleoside phosphorylase (*pdp*) from *Bacillus subtilis* were cloned, and their nucleotide sequences were determined. Sequence analysis showed that the genes were localized immediately downstream of the *hut* operon. Insertional gene disruption studies indicated that the three genes constitute an operon with the gene order *dra-nupC-pdp*. A promoter mapping immediately upstream of the *dra* gene was identified, and downstream of the *pdp* gene the nucleotide sequence indicated the existence of a factor-independent transcription terminator structure. In wild-type cells growing in succinate minimal medium, the pyrimidine nucleoside phosphorylase and deoxyriboaldolase levels were five- to eightfold higher in the presence of thymidine and fourfold higher in the presence of deoxyadenosine. By the use of *lacZ* fusions, the regulation was found to be at the level of transcription. The operon expression was subject to glucose repression. Upstream of the *dra* gene an open reading frame of 313 amino acids was identified. Inactivation of this gene led to an approximately 10-fold increase in the levels of deoxyriboaldolase and pyrimidine nucleoside phosphorylase, and no further induction was seen upon the addition of deoxyribonucleosides. The upstream gene most likely encodes the regulator for the *dra-nupC-pdp* operon and was designated *deoR* (stands for deoxyribonucleoside regulator).

Pyrimidine deoxyribonucleosides are catabolized by many bacteria, including the gram-positive soil bacterium *Bacillus subtilis*. The catabolic pathway consists of three major steps: (i) uptake, (ii) phosphorolytic cleavage, and (iii) degradation of the pentose moiety. After transport into the *B. subtilis* cell, pyrimidine deoxyribonucleosides are cleaved by pyrimidine nucleoside phosphorylase to deoxyribose-1-phosphate (dRib-1-P) and the pyrimidine base. The pyrimidine base can be returned to the nucleotide pool via the pyrimidine salvage reactions (21). dRib-1-P can be converted to deoxyribose-5-phosphate (dRib-5-P) in a reaction catalyzed by phosphodeoxyribomutase. Finally, dRib-5-P is cleaved to acetaldehyde and glyceraldehyde-3-phosphate by the action of deoxyriboaldolase. In *B. subtilis* the *pdp* and *dra* genes, which encode pyrimidine nucleoside phosphorylase and deoxyriboaldolase, respectively, are closely linked on the chromosome at 339° on the linkage map. The *drm* gene encoding phosphodeoxyribomutase is located at 182° on the linkage map (22). In *Escherichia coli* the genes encoding deoxyriboaldolase (*deoC*), phosphodeoxyribomutase (*deoB*), and thymidine phosphorylase (*deoA*) are organized in the *deo* operon (9). In addition to these genes, the *deo* operon also contains the *deoD* gene, which encodes purine nucleoside phosphorylase. The regulation of expression of the *E. coli deo* operon has been studied in great detail. The expression is controlled by the DeoR and CytR repressor proteins and by the cyclic AMP (cAMP)-cAMP receptor protein complex (cAMP-CRP) (6, 20, 28). *E. coli* grows well with deoxyribonucleosides as the sole carbon and energy source. The addition of deoxyribonucleosides induces *deo* operon expression through the intracellular formation of dRib-5-P, which is the effector for the DeoR repressor (8, 18). Although *B. subtilis* has the capacity to degrade deoxyribonucleosides, these compounds function only poorly as carbon and energy sources. This can be explained by limited enzyme induction by deoxyribonucleosides or by inefficient nucleoside transport (9). The levels
of pyrimidine nucleoside phosphorylase, phosphodeoxyribomutase, and deoxyriboaldolase are induced by the presence in the growth medium of deoxyribonucleosides such as thymidine (22). dRib-5-P and, also, another unidentified compound are believed to act as the true inducers of *dra*, *pdp*, and *drm* expression (22). The existence of at least three different nucleoside uptake systems in *B. subtilis* has been reported. One is specific for hypoxanthine and guanine nucleosides, another is specific for adenine nucleosides, and a third is specific for the transport of cytidine and uridine and the corresponding deoxyribonucleosides (1, 7, 12). In *E. coli*, nucleoside uptake is mediated by two membrane proteins encoded by the *nupC* and *nupG* genes. The NupG protein transports all ribonucleosides and deoxyribonucleosides, and the NupC protein transports all nucleosides except hypoxanthine and guanine nucleosides (5, 19).

In this report, we show that in *B. subtilis* the *dra*, *nupC*, and *pdp* genes are organized in an operon. We also report the identification of a gene (*deoR*) that encodes a negative regulator of expression of the *dra-nupC-pdp* operon.

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MATERIALS AND METHODS

Bacterial strains, plasmids, and media. The bacterial strains and plasmids used in this work are listed in Table 1. The λ genome library was kindly provided by C. W. Price. *B. subtilis* was grown in Spizizen minimal salt medium (27), and

TABLE 1. Bacterial strains and plasmids[a]

Strain or plasmid	Genotype or description	Source or reference
Strains		
B. subtilis		
168	*trpC2*	C. Anagnostopoulos
LA804	*trpC2 pdp*::pLNA124 (Cm^r)	Tf[b] of 168 by pLNA124, Cm^r
LA878	*trpC2 nupC*::pLNA138 (Cm^r)	Tf of 168 by pLNA138, Cm^r
HH218	*trpC2 amyE*::*deoR-dra-lacZ* (Neo^r)	Tf of 168 by pHH1015 digested with *Xho*I, Neo^r
HH219	*trpC2 amyE*::*dra-lacZ* (Neo^r)	Tf of 168 by pHH1014 digested with *Xho*I, Neo^r
HH221	*trpC2 dra*::pHH1001 (Cm^r)	Tf of 168 by pHH1001, Cm^r
HH229	*trpC2 dra*::pHH1011 (Cm^r)	Tf of 168 by pHH1011, Cm^r
HH232	*trpC2 deoR*::*erm* (Er^r)	Tf of 168 by pHH1019 digested with *Hin*dIII, Er^r
HH234	*trpC2 dra*::*erm* (Er^r)	Tf of 168 by pHH1022 digested with *Hin*dIII, Er^r
HH235	*trpC2 deoR*::*erm* (Er^r) *amyE*::*deoR-dra-lacZ* (Neo^r)	Tf of HH232 by HH218, Neo^r
HH236	*trpC2 deoR*::*erm* (Er^r) *amyE*::*dra-lacZ* (Neo^r)	Tf of HH232 by HH219, Neo^r
HH239	*trpC2 amyE*::pHH1024 (Neo^r Er^r)	Tf of 168 by pHH1024 digested with *Xho*I, Neo^r and Er^r
HH240	*trpC2 amyE*::pHH1025 (Neo^r Er^r)	Tf of 168 by pHH1025 digested with *Xho*I, Neo^r and Er^r
E. coli		
SØ930	*deoR clmA* Δ*deo* Δ*lac thi udp upp ton*	30
LA5	*thyA deoR clmA* Δ*deo* Δ*lac thi udp upp ton*	This work
MT102	F^-/*araD139* Δ(*argF-leu*)*7696* Δ(*lac*)*X74 galU galK hsdR2* (r^- m^+) *mcrB1 rpsL* (Str^r)	Laboratory stock
DH5α	F′/*endA1 hsdR17* (r^- m^-) *supE44 thi-1 recA1 gyrA* (Nal^r) *relA1* Δ(*lacZYA-argF*)*U169 deoR* (ϕ80 *dlac*Δ(*lacZ*)M15)	Laboratory stock
Plasmids		
pBOE335	Ap^r Cm^r	14
pBR322	Ap^r Tc^r	Laboratory stock
pSGMU38	Ap^r Cm^r	J. Errington
pKS+	Ap^r	Stratagene
pUC7*erm*	Ap^r Er^r	W. de Vos
pDG268*neo*	Ap^r Neo^r	C. Price
pLNA2	Ap^r Tc^r Cm^r	26
pLNA6	2.9-kb *Eco*RI fragment of a λgt11 clone containing *pdp* ligated to pLNA2 digested with *Eco*RI	This work
pLNA11	3.6-kb *Eco*RI fragment of a λgt11 clone containing *nupC* and *pdp* ligated to pLNA2 digested with *Eco*RI	This work
pLNA14	Like pLNA6, but with the insert in the opposite orientation	This work
pLNA93	2-kb *Nru*I-*Bgl*II fragment flanking the *pdp* gene ligated to pHH600 digested with *Hin*dIII and *Bam*HI; the *Hin*dIII site was blunt ended in a Klenow polymerase reaction prior to ligation; transcriptional *lacZ* fusion	This work
pLNA124	987-bp *Hin*dIII-*Bam*HI fragment from pLNA11 containing an internal part of *pdp* ligated to pHH601 digested with *Hin*dIII and *Bam*HI; translational *pdp-lacZ* fusion	This work
pLNA138	495-bp *Hin*dIII-*Hin*cII fragment from pLNA11 containing an internal part of *nupC* ligated to pHH601 digested with *Hin*dIII and *Sma*I; transcriptional *nupC-lacZ* fusion	This work
pHH600	4-kb *Hin*dIII-*Bgl*II fragment from pSGMU38 containing *lac* and *cat* ligated to pBR322 digested with *Hin*dIII and *Bam*HI	This work
pHH601	Insertion of a *Sma*I linker into pHH600 which has been digested with *Bam*HI and treated with Klenow polymerase	This work
pHH1001	300-bp *Eco*RI-*Sph*I fragment from pLNA11 containing an internal part of *dra* ligated to pBOE335 digested with *Eco*RI and *Sph*I	This work
pHH1002	pBOE335 derivative containing the *dra* 5′ end and 1.5 kb of upstream DNA; obtained by the plasmid rescue technique using religated DNA from HH221 digested with *Eco*RI	This work
pHH1011	760-bp *Pvu*II-*Hin*cII fragment from pHH1002 containing the 3′ end of *deoR* and 5′ end of *dra* ligated to pHH601 digested with *Sma*I; translational *dra-lacZ* fusion	This work
pHH1012	780-bp *Sma*I fragment from pHH1002 containing the 5′ end of *deoR* ligated to pHH601 digested with *Sma*I; transcriptional *deoR-lacZ* fusion	This work
pHH1014	1.1-kb *Sma*I-*Hin*dIII fragment from pHH1002 containing the 3′ end of *deoR* and the 5′ end of *dra* ligated to pDG268*neo* digested with *Eco*RI and *Hin*dIII; the *Eco*RI ends were treated with Klenow polymerase prior to digestion with *Hin*dIII and subsequent ligation; transcriptional *dra-lacZ* fusion	This work
pHH1015	1.9-kb *Eco*RI-*Hin*dIII fragment from pHH1002 containing the *deoR* gene and the 5′ end of *dra* ligated to pDG268*neo* digested with *Eco*RI and *Hin*dIII; transcriptional *dra-lacZ* fusion	This work

Continued on following page

TABLE 1—*Continued*

Strain or plasmid	Genotype or description	Source or reference
pHH1016	Deletion of an 820-bp *Stu*I fragment from pHH1002	This work
pHH1017	1.1-kb *Eco*RI fragment from pUC7*erm* containing *erm* ligated to pKS+ digested with *Eco*RI	This work
pHH1019	1.1-kb *Sma*I-*Eco*RV fragment from pHH1017 containing *erm* ligated to pHH1016 digested with *Sma*I; insertion of *erm* into *deoR*	This work
pHH1022	1.1-kb *Sma*I-*Eco*RV fragment from pHH1017 containing *erm* ligated to pHH1016 digested with *Hinc*II; insertion of *erm* into *dra*	This work
pHH1024	1.1-kb *Eco*RI fragment from pHH1017 containing *erm* ligated to pDG268*neo* digested with *Eco*RI; antiparallel orientation of *erm* and *lacZ*	This work
pHH1025	Like pHH1024; parallel orientation of *erm* and *lacZ*	This work

[a] Ap, ampicillin; Cm, chloramphenicol; Tc, tetracycline; Er, erythromycin; Neo, neomycin; Str, streptomycin; Nal, nalidixic acid.
[b] Tf, transformation of the first strain either with the noted plasmid or with DNA from the second strain.

E. coli was grown in a phosphate-buffered salt medium (17). Both minimal media were supplemented with 1 μg of thiamine hydrochloride per ml. *B. subtilis* minimal medium was supplemented with 50 μg of L-tryptophan per ml and with either 0.4% glucose or 0.4% succinate. Additional amino acids required by auxotrophic strains were added at 50-μg/ml concentrations. Thymine (20 μg/ml) and thymidine (20 μg/ml) were added to thymine- and thymidine-requiring *E. coli* mutants, respectively. L broth (Difco Laboratories, Detroit, Mich.) was used as a rich medium for both *E. coli* and *B. subtilis*. Unless otherwise stated, culturing of cells was performed at 37°C. For selection of antibiotic resistance, antibiotics were used at the following concentrations: ampicillin (100 μg/ml), chloramphenicol (5 μg/ml), neomycin (5 μg/ml), erythromycin (150 μg/ml) (*E. coli*); erythromycin and lincomycin (1 and 25 μg/ml, respectively) (*B. subtilis*). *E. coli thyA* mutants were selected on minimal medium agar plates containing 8 μg of trimethoprim and thymidine per ml.

DNA manipulations and genetic techniques. Chromosomal DNA from *B. subtilis* (27) and recombinant λ DNA were isolated as described previously (23). Plasmid DNA was isolated by the alkaline-sodium dodecyl sulfate method (2). Transformation of *E. coli* (10) and *B. subtilis* (3) and λ-mediated transduction of *E. coli* (23) were performed as previously described. Treatment of DNA with restriction enzymes, T4 DNA ligase, and Klenow polymerase was performed as recommended by the supplier (Gibco BRL, Gaithersburg, Md.). For Southern blot analysis, DNA was blotted onto a nitrocellulose membrane (GeneScreen; Dupont, NEN Research Products, Boston, Mass.). The DIG DNA Labelling and Detection Kit (Boehringer, Mannheim, Germany) was used for colorimetric detection of the hybridization products. The recipe supplied by the manufacturer was followed.

DNA sequencing. DNA sequences were obtained by the chain-termination reaction method with dideoxyribonucleotides as described by Sanger et al. (24). All sequencing analysis was done on double-stranded plasmid DNA templates.

Isolation of RNA and primer extension analysis. Total RNA from *B. subtilis* was isolated by a previously described method (26). The primer extension analysis using Superscript RNaseH$^-$ reverse transcriptase (Gibco BRL) has also been described previously (26). The radiolabelled cDNA products were separated on a 6% polyacrylamide sequencing gel and visualized by autoradiography.

Enzyme assays. Cell extracts were made by disrupting cells suspended in MOPS (morpholinepropanesulfonic acid) buffer (50 mM MOPS [pH 7.1], 1 mM EDTA, 1 mM dithiothreitol) by sonic treatment at 0°C for 1 min. Cell debris was removed by centrifugation, and the extracts were dialyzed for 18 h at 5°C against dialyzing buffer (10 mM MOPS [pH 7.6], 50 μM EDTA). Pyrimidine nucleoside phosphorylase activity with thymidine as the substrate was determined in an assay modified from one previously performed with extracts of *E. coli* (30). A total volume of 350 μl of assay mixture (14.3 mM phosphate [pH 7.1], 40 mM MOPS [pH 7.1], 0.8 mM EDTA, 0.8 mM dithiothreitol, and suitable amounts of cell extract) was incubated for 2 min at 37°C. The reaction was started by adding thymidine (final concentration, 5.7 mM), and after 0.5, 10, and 20 min, 100-μl samples were pipetted into 200 μl of 0.15 M perchloric acid. The dRib-1-P concentration was determined with thiobarbituric acid (25). Deoxyriboaldolase activity was determined with an assay modified from a previously described method (20). A total of 200 μl of assay mixture (55 mM MOPS [pH 7.4], 2 mM EDTA, 1.5 mM NADH, 60 μg of alcohol dehydrogenase per ml, and suitable amounts of cell extract) was incubated for 2 min at 37°C. The reaction was started by adding dRib-5-P (final concentration, 1 mM), and after 0.5, 10, and 20 min, 50-μl samples were pipetted into 400 μl of 7% perchloric acid. The dRib-5-P concentration was determined with diphenylamine (4). Deoxyribomutase activity was determined with an assay modified from one previously evolved for *E. coli* (8). A total of 100 μl of assay mixture (1.4 μM ribose-1,5-diphosphate, 0.2 mM $CoCl_2$, 57 mM MOPS [pH 7.6], and suitable amounts of cell extract) was incubated for 10 min at 0°C. The reaction was started by adding dRib-1-P (final concentration, 0.4 mM), and the reaction mixture was incubated at 37°C. After 0.5, 10, and 20 min, 25-μl samples were pipetted into 275 μl of 0.15 M perchloric acid. The dRib-1-P concentration was determined with thiobarbituric acid (25). β-Galactosidase activity was measured according to the method of Miller (16). Specific enzyme activities were expressed in units per milligram of protein. One unit is defined as 1 nmol of substrate converted per min. The enzyme analysis was performed in triplicate, and the presented values are means of at least three different experiments. Total protein was determined by the method of Lowry et al. (13). Detection of α-amylase activity in *B. subtilis* cells was performed by adding a solution of 0.5% iodine and 1% potassium iodine to colonies grown on L-broth agar plates containing 1% starch. α-Amylase-producing colonies formed a clear halo, whereas α-amylase-negative colonies did not.

Measurement of nucleoside uptake and incorporation. *B. subtilis* cells were grown exponentially in glucose minimal medium. At an optical density at 450 nm equal to 0.5 to 0.7, 10 ml of culture was filtered, washed with 25 ml of prewarmed medium, and resuspended in 10 ml of fresh prewarmed medium. After 5 min of incubation, 1.5 ml was transferred to a plastic vial containing 15 μl of 0.1 mM ^{14}C-labelled nucleoside (0.2 GBq/mmol). Incorporation into nucleic acid was monitored over an 8-min period by sampling 200 μl into 5 ml of 5% ice-cold trichloroacetic acid. After 30 min at 0°C, the samples were filtered and the filter-bound radioactivity was determined by electronic autoradiography in a Pachard Instant Imager. For short-term uptake measurement, 1 ml of the filtered and resuspended culture was transferred to a plastic tube containing 10 μl of the same isotopes as those used in the incorporation experiment. Four samples of 200 μl were withdrawn within 1 min and immediately filtered and washed with 5 ml of isotope-free minimal medium. Radioactivity bound to the filters was determined as described above. Incorporation and uptake rates were expressed in nanomoles per minute per milligram (dry weight). One milliliter of culture at an optical density at 450 nm of 1.0 contains 0.2 mg (dry weight) of cells.

Nucleotide sequence accession number. The nucleotide sequence of the overlapping segments of *B. subtilis* genomic DNA cloned in the plasmids pLNA11 and pHH1002 has been deposited in the GenBank data bank with the accession number X82174.

RESULTS

Induction by deoxyribonucleosides and glucose repression. Deoxyriboaldolase and pyrimidine nucleoside phosphorylase activities were determined in wild-type strain 168 grown in minimal medium with glucose or succinate as the carbon and energy source (Table 2). The addition of thymidine or deoxyadenosine to cells growing in succinate minimal medium resulted in 4.3- to 8.8-fold increases in enzyme levels. In general, the enzyme levels were two- to sevenfold higher in succinate-grown cells than in glucose-grown cells. The addition of inosine, cytidine, or uridine did not increase the enzyme levels in either glucose- or succinate-grown cells (data not shown). We conclude that levels of deoxyriboaldolase and pyrimidine nucleoside phosphorylase are induced in the presence of deoxyribonucleosides but not in the presence of ribonucleosides and that they are repressed by glucose.

Cloning of the *deoR-dra-nupC-pdp* gene cluster. *E. coli* LA5 (*thyA* Δ*deo udp*) was isolated among trimethoprim-resistant clones of strain SØ930 (Δ*deo udp*) (Table 1). Because of the *thyA* mutation and the lack of thymidine phosphorylase activity in the cell (*deoA udp*), LA5 required thymidine for growth.

TABLE 2. Levels of deoxyriboaldolase and pyrimidine nucleoside phosphorylase in wild-type *B. subtilis* 168 and effects of carbon source and of different inducers[a]

Carbon source	Inducer added[b]	Enzyme[c] activity (nmol/min/mg)	
		dra	*pdp*
Glucose	None	35	15
	TdR	104 (3.0)[d]	45 (3.0)
	AdR	120 (3.4)	38 (2.5)
Succinate	None	46	34
	TdR	240 (5.2)	301 (8.8)
	AdR	198 (4.3)	161 (4.7)

[a] Cells were grown in minimal medium supplemented with the stated carbon source. Inducers were added to a final concentration of 1 mg/ml.
[b] TdR, thymidine; AdR, deoxyadenosine.
[c] Enzymes are represented by their gene designation.
[d] Numbers in parentheses indicate fold of regulation.

LA5 was transduced with a phage library containing random *B. subtilis* genomic *Alu*I, *Hae*III, and *Rsa*I restriction fragments attached to *Eco*RI linkers and ligated to the *Eco*RI site of λgt11. To isolate the *B. subtilis pdp* gene, lysogens were selected at 30°C on glucose minimal medium agar plates containing thymine. The presence of thymidine phosphorylase encoded by *pdp* allowed the *thyA* Δ*deo* mutant to grow with thymine as the thymidine source. Forty lysogens were obtained. Among these, four did not require thymine (possibly one of the *thy* genes from *B. subtilis* was cloned). The rest were able to grow when thymine was provided and were resistant to trimethoprim, as expected for lysogens containing a *pdp* gene complementing the Δ*deo* mutation. Maznitsa (15) previously cloned the *pdp* gene from *B. subtilis* by complementation of an *E. coli deoA udp* strain. These *pdp* clones showed a uridine phosphorylase/thymidine phosphorylase activity ratio of 0.6 to 0.9. Maznitsa also isolated clones of a *deoA* type with a uridine/thymidine ratio of 0.01 to 0.02 (15). Therefore, the phosphorylase activity towards uridine and thymidine was measured in extracts of the 36 Pdp^+ lysogens isolated in this work. They all showed a uridine phosphorylase/thymidine phosphorylase activity ratio of 0.4 to 0.8, indicating that no clones of the *deoA* type were obtained. Recombinant λ DNA from four lysogens was isolated. One clone had a 4.7-kb insert; one clone contained a 3.6-kb insert; and two clones appeared identical, with an insert of 2.9 kb. The 3.6- and 2.9-kb fragments were subcloned into plasmid pLNA2, yielding pLNA11 (3.6 kb) and pLNA6 and pLNA14 (2.9 kb) (Fig. 1A). Restriction analysis showed that the 2.9-kb insert constituted an internal part of the 3.6-kb fragment (Fig. 1A). All three plasmids complemented the *deoA* mutation of LA5, when tested on minimal agar plates containing thymine. However, LA5 harboring plasmid pLNA6 or pLNA11 contained 0.25 U of pyrimidine nucleoside phosphorylase activity per mg of protein, whereas LA5 with pLNA14 contained 21 U/mg of protein (Fig. 1A). As described later, sequence analysis revealed that the 3.6-kb fragment contained the *pdp* and *nupC* genes and the 3′ end of the *dra* gene.

A standard plasmid rescue technique was used to isolate DNA upstream of *dra* (33). A 300-bp *Eco*RI-*Sph*I internal *dra* fragment was subcloned into plasmid pBOE335, creating pHH1001. pHH1001 was introduced into *B. subtilis* 168 selecting Cm^r. Since pBOE335 derivatives cannot replicate in *B. subtilis*, pHH1001 integrated into the chromosome at the site of homology, in this case the *dra* gene. Southern blot analysis confirmed that the correct integration of plasmids and antibiotic resistance cassettes had occurred. Plasmid pHH1002 containing the *dra* 5′ end and 1.5 kb of upstream DNA was isolated by restricting chromosomal DNA of strain HH221 [*dra*::pHH1001 (Cm^r)] with *Eco*RI; this was followed by religation and subsequent transformation of *E. coli* MT102.

Nucleotide and derived amino acid sequence and similarity to other sequences. The nucleotide sequence of the overlapping segments of *B. subtilis* genomic DNA cloned in the plasmids pLNA11 and pHH1002 was determined and is shown in Fig. 2. The sequence contained four open reading frames all transcribed in the same direction. The choice of translational

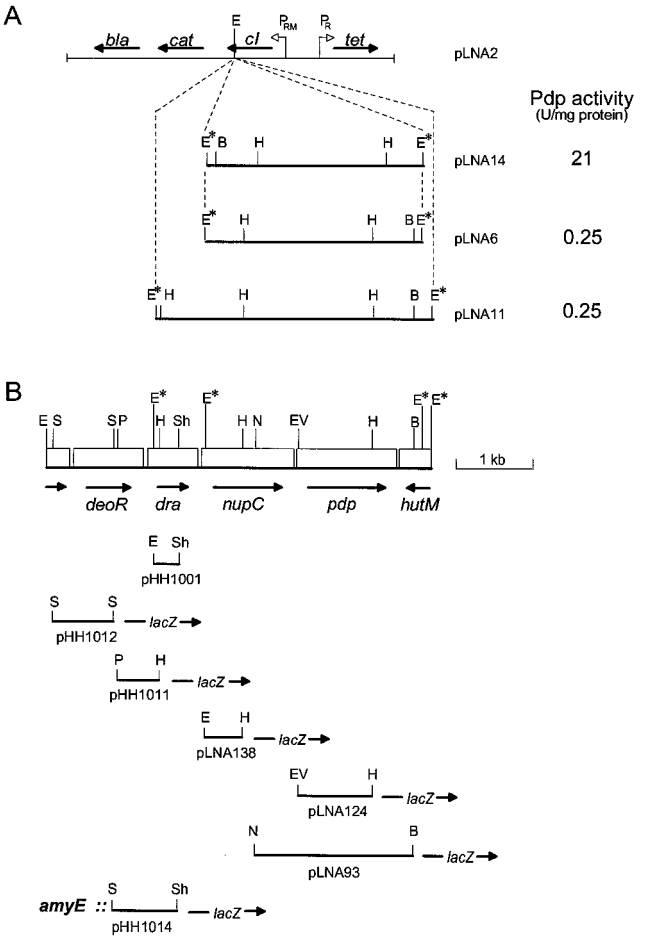
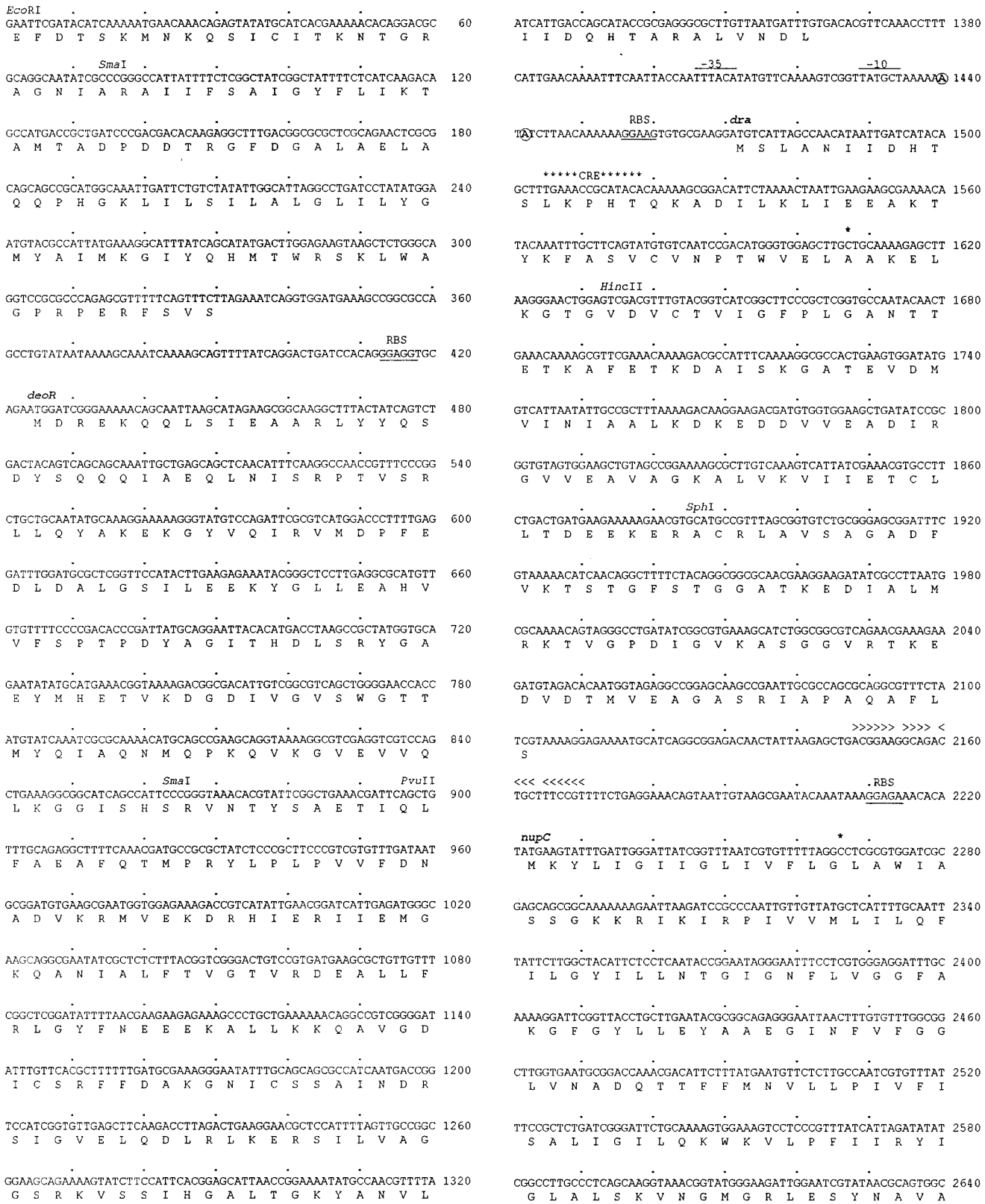


FIG. 1. Physical map of plasmid pLNA2 and of the *deoR-dra-nupC-pdp* region of *B. subtilis*. (A) The lines beneath the map of the cloning vector pLNA2 show the fragments cloned into the *Eco*RI site. These pLNA2 constructions were transformed into *E. coli* LA5 (*thyA* Δ*deo udp*), and the pyrimidine nucleoside phosphorylase (Pdp) activity of the transformants was measured as described in Materials and Methods. (B) The lines beneath the map show the different fragments cloned into the single-copy *lacZ*-fusion vectors pHH600, pHH601, and pDG268*neo* (pHH1014). Closed circular plasmid molecules of the pHH600 and pHH601 derivatives were integrated into the *deoR-dra-nupC-pdp* region by transformation. The pDG268*neo* derivative pHH1014 was linearized and transformed into the *amyE* locus of *B. subtilis*. Arrows indicate the location and direction of transcription of the different genes and open reading frames. Symbols: thick line, *B. subtilis* DNA; thin line, plasmid DNA; open block, *B. subtilis* gene; *bla*, β-lactamase; *cat*, chloramphenicol acetyltransferase; *tet*, tetracycline resistance gene; *deoR*, deoxyribonucleoside repressor protein; *dra*, deoxyriboaldolase; *nupC*, nucleoside transport protein; *pdp*, pyrimidine nucleoside phosphorylase; *hutM*, putative histidine permease; *lacZ*, β-galactosidase; *amyE*, α-amylase; P_{RM}, λ P_{RM} promoter; P_R, λ P_R promoter; E, *Eco*RI site in pLNA2 and the one native to the *B. subtilis* genome; B, *Bgl*II; H, *Hin*cII; S, *Sma*I; P, *Pvu*II; Sh, *Sph*I; N, *Nru*I; EV, *Eco*RV; E*, position of *Eco*RI linkers inserted during library construction.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SmaI
RBS
deoR
SmaI
PvuII
HincII
SphI
RBS.
dra
-35
-10
*****CRE******
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.RBS

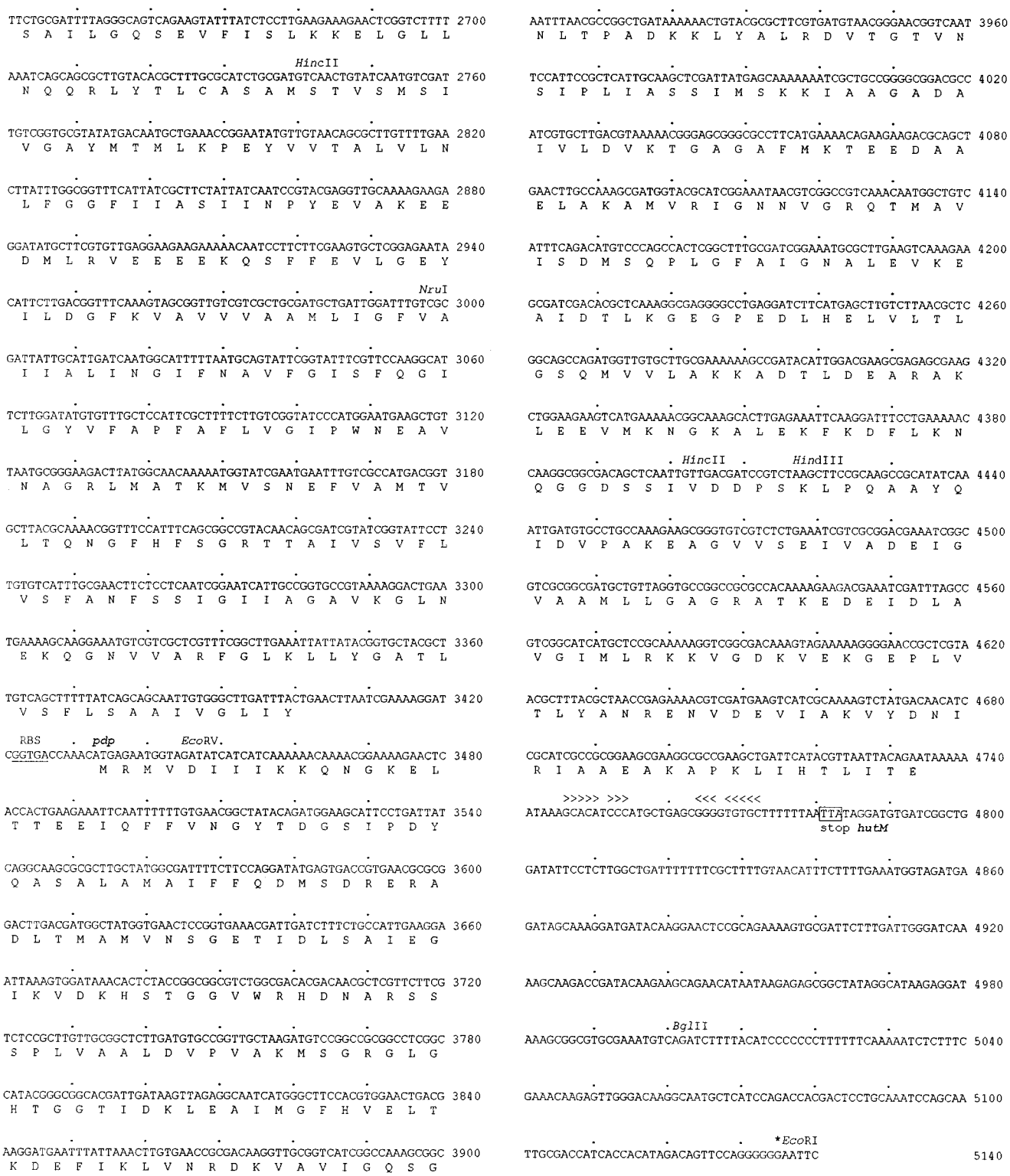

FIG. 2. Nucleotide and deduced amino acid sequence of the *B. subtilis deoR-dra-nupC-pdp* gene cluster. Underlined sequence indicates putative ribosome binding sites (RBS). Lines above the nucleotide sequence show the putative −35 and −10 regions of the *dra-nupC-pdp* operon promoter. Circled nucleotides at position 1440 and 1442 show the start points of the *dra-nupC-pdp* transcript. The position of inverted repeated sequences in the *dra-nupC* intercistronic region and downstream of *pdp* are shown (>>><<<). A putative CRE (11) at positions 1505 to 1518 is indicated by asterisks above the sequence. Boxed nucleotides at positions 4781 to 4783 indicate the complementary sequence of the *hutM* translational stop signal. Boldface asterisks above nucleotides 1607, 2276, and 5135 show the positions of the *Eco*RI linkers used in the library construction. Only relevant restriction sites are shown.

TABLE 3. Levels of deoxyriboaldolase, pyrimidine nucleoside phosphorylase, and β-galactosidase in *B. subtilis* strains carrying different plasmid or *erm* cassette integrations in the *deoR-dra-nupC-pdp* gene cluster[a]

Strain	Relevant genotype	Inducer added[b]	Enzyme[c] activity (nmol/min/mg)		
			dra	*pdp*	*lacZ*
HH229	*dra*::pHH1011 *lacZ*$^+$	None	37	31	4
		TdR	216 (5.9)[d]	301 (9.6)	20 (5.0)
		AdR	ND[e]	ND	ND
LA878	*nupC*::pLNA138 *lacZ*$^+$	None	38	3	5
		TdR	78 (2.1)	3 (0.8)	19 (4.2)
		AdR	149 (3.9)	3 (1.0)	21 (4.5)
LA804	*pdp*::pLNA124 *lacZ*$^+$	None	33	<0.1[f]	40
		TdR	37 (1.1)	<0.1	38 (1.0)
		AdR	202 (6.1)	2	258 (6.4)
HH221	*dra*::pHH1001 Δ*lacZ*	None	42	<0.1	2
		TdR	60 (1.4)	<0.1	2 (1.0)
		AdR	160 (3.8)	<0.1	1 (0.8)
HH234	*dra*::*erm* Δ*lacZ*	None	0.0	786	ND
		TdR	0.0	597	ND
		AdR	0.0	714	ND
HH230	*deoR*::pHH1012 *lacZ*$^+$	None	36	26	2
		TdR	209 (5.7)	174 (6.7)	2 (1.0)
		AdR	161 (4.4)	129 (5.0)	1 (0.7)
HH232	*deoR*::*erm* Δ*lacZ*	None	470	597	ND
		TdR	405	430	ND
		AdR	563	556	ND

[a] Cells were grown in succinate minimal medium. Inducers were added to a final concentration of 1 mg/ml.
[b] TdR, thymidine; AdR, deoxyadenosine.
[c] Enzymes are represented by their gene designations.
[d] Numbers in parentheses indicate fold of regulation.
[e] ND, not determined.
[f] Uridine phosphorylase activity was also lost in LA804.

start codon for these reading frames was based on the positions of potential Shine-Dalgarno sequences and on their homology to known genes. The first reading frame (designated *deoR*) encoded a protein of 314 amino acids with 30% identity to the SorC repressor protein of *Klebsiella pneumoniae* (GenBank accession number X66059), the second reading frame of 246 codons (designated *dra*) showed 48% identity to the *deoC*-encoded deoxyriboaldolase of *Mycoplasma pneumoniae* (GenBank accession number X13544), the third open reading frame of 394 codons (designated *nupC*) was 55% identical to the NupC nucleoside transport protein of *E. coli* (5), and the last reading frame of 435 codons (designated *pdp*) was 42% identical to the *deoA*-encoded thymidine phosphorylase of *E. coli* (31). A sequence with dyad symmetry between the *dra* and *nupC* genes was found, and a potential factor-independent transcription terminator structure is located immediately downstream of the translational stop signal of the *pdp* gene. The sequence from nucleotide 4417 (the *Hin*dIII site in the 3′ end of the *pdp* gene) to 5134 was identical to the first 718 nucleotides of a 29-kb *B. subtilis* nucleotide sequence which encodes the *hut* operon and several other genes (32). The *hutM* gene is encoded by the opposite strand of that of the *pdp* gene. The translational stop signal of the *hutM* gene (encoding a histidine permease) is at position 4781, immediately downstream of the putative factor-independent transcription terminator sequence. This potential stem and loop may be capable of terminating transcription from both directions.

Characterization of mutants in the *dra*, *nupC*, and *pdp* genes. Plasmids pHH1001, pLNA138, and pLNA124, each containing an internal part of the *dra*, *nupC*, and *pdp* genes, respectively, were constructed, and plasmid integration into the chromosome of *B. subtilis* resulted in the following strains: LA878, LA804, and HH221. These, as well as all other used integrants, were verified by Southern blotting. The activities of deoxyriboaldolase and pyrimidine nucleoside phosphorylase in these mutants are presented in Table 3. The levels may be compared with those found in HH229. Strain HH229 contains the plasmid pHH1011 integrated into the *dra* locus. As described later, pHH1011 carries the region containing the promoter, therefore allowing for downstream transcription of *nupC* and *pdp*. Thus, enzyme levels in strain HH229 are identical to the wild-type levels shown in Table 2. Strain LA804 (*pdp*::pLNA124) had lost pyrimidine nucleoside phosphorylase activity both when thymidine was used as the substrate (Table 3) and when uridine was used as the substrate. This result shows that the *pdp* gene encodes the only pyrimidine phosphorylase found in *B. subtilis*. In contrast to this result we found that strain HH221 (*dra*::pHH1001) still contained deoxyriboaldolase activity. Since HH221 contains 80% of the *dra* gene, a second gene inactivation was introduced in the first quarter part of the *dra* gene. HH234 was constructed by insertion of the *erm* gene into the unique *Hin*cII site in the *dra* gene (Table 1 and Fig. 2). This insertion resulted in the loss of deoxyriboladolase activity, as seen in Table 3. This result shows

TABLE 4. Rate of uptake and incorporation of ^{14}C-labelled uridine and inosine by wild-type *B. subtilis* and various mutant strains[a]

Strain	Relevant genotype	Rate (nmol/min/mg [dry wt])			
		Uptake		Incorporation	
		[^{14}C]UR[b]	[^{14}C]IR[c]	[^{14}C]UR	[^{14}C]IR
168	Wild type	0.96	1.12	0.43	0.48
LA878	*nupC*::pLNA138	0.21	1.25	0.10	0.48
LA804	*pdp*::pLNA124	0.74	0.87	0.40	0.40

[a] Cells were grown in glucose minimal medium.
[b] [^{14}C]UR, ^{14}C-labelled uridine.
[c] [^{14}C]IR, ^{14}C-labelled inosine.

that *dra* encodes the only deoxyriboaldolase found in *B. subtilis* under the physiological condition tested. We have not analyzed deoxyriboladolase activity during stationary phase or sporulation. The expected phenotype of a *nupC* mutant in *B. subtilis* would be loss of the ability to transport the pyrimidine nucleosides, but not the purine nucleoside inosine, provided that the system is analogous to that found in *E. coli*. Table 4 shows the results of uptake into washed cells within 1 min and of incorporation of radiolabelled uridine and inosine into the acid-precipitable cellular components. Three strains were used: the wild-type 168 and two mutant strains, LA878 (*nupC*::pLNA138) and LA804 (*pdp*::pLNA124). LA804, having no pyrimidine nucleoside phosphorylase activity, was included as a more appropriate control than 168 for the experiments with the *nupC* mutant, LA878, since this mutant has very low levels of pyrimidine nucleoside phosphorylase because polarity and splitting of the nucleoside inside the cell might increase the rate of uptake and/or incorporation. The rates of both uptake and incorporation of uridine were four times lower in the *nupC* integrant than in the *pdp* control strain. This result clearly shows that *nupC* encodes a protein involved in transport of the pyrimidine nucleoside uridine and that the *nupC*-encoded system is the main transport system for uridine in *B. subtilis*. No reduction in inosine transport was found in the *nupC* mutant. The results also show that the incorporation of uridine into nucleic acids was not affected by the *pdp* mutation.

Evidence for an operon structure. If the *dra-nupC-pdp* gene cluster constitutes an operon, the gene disruptions described above should result in a polarity effect on expression of any gene located in the operon downstream of the insertion. To investigate this possibility, the level of the gene product of the downstream gene *pdp* was measured in the strains LA878 (*nupC*::pLNA138) and HH221 (*dra*::pHH1001), and as shown in Table 3, the pyrimidine nucleoside phosphorylase activity was absent or present in very low levels in both of these mutant strains. These results indicate that the gene cluster *dra-nupC-pdp* is transcribed from a promoter located upstream of the *dra* gene. This conclusion is not violated by the high level of pyrimidine nucleoside phosphorylase found in HH234 (*dra*::*erm*), since this was shown to be due to readthrough from the *erm* promoter in the following way: the *erm* cassette was cloned in front of the *lacZ* gene in plasmid pDG268, and this was followed by recombination into the chromosomal *amyE* locus. Parallel direction of transcription of the *erm* and *lacZ* genes resulted in 88 U of β-galactosidase activity per mg, while cloning in the opposite direction yielded 1.6 U/mg. Thus, substantial amounts of transcription extends out of the *erm* cassette into any gene located downstream. This explains the high levels of *pdp* found in HH234, since the *erm* promoter is oriented towards the *pdp* gene in this mutant.

In order to locate the promoter upstream of *dra*, plasmid pHH1011 containing the DNA sequence from the 3′ end of *deoR* to the *Hin*cII site in the 5′ end of the *dra* gene was integrated into the chromosome, resulting in strain HH229. If a promoter is located within the insert of pHH1011, no effect of polarity on *pdp* should be observed, while if the cloned fragment contains only an internal part of the operon, an effect of polarity on the expression of the genes downstream of the insertion point should be seen. The results shown in Table 3 show the presence of wild-type, inducible deoxyriboaldolase and pyrimidine nucleoside phosphorylase levels in HH229; thus, the thymidine-inducible promoter is located within the *Pvu*II-*Hin*cII fragment cloned in pHH1011.

The end of the operon was located by chromosomal insertion of plasmid pLNA93 containing the putative transcription terminator following the *pdp* gene (Fig. 1). This plasmid, like most of the previously used plasmids, contains the *lacZ* gene just after the cloning sites; therefore, the insertion will result in transcriptional fusions of the upstream chromosomal regions, including the cloned sequence, to *lacZ*, the exception being one particular reading frame, where translational fusions may arise (Table 1). The strain resulting from the integration event (LA544) exhibited β-galactosidase levels below detectable levels even after induction with thymidine (data not presented), showing that a transcriptional terminator is present within the cloned fragment in pLNA93. The precise location of the promoter was determined by primer extension. Two primers complementary to the nucleotide sequences 1525 to 1546 (primer 1) and 1669 to 1684 (primer 2), respectively, were used. Figure 3 shows the result of a primer extension experiment using primer 2. The same primer was used to generate the sequence ladder. RNA (20 μg) from cells of strain 168 grown in the absence (Fig. 3, lane 1) or in the presence (lane 2) of thymidine

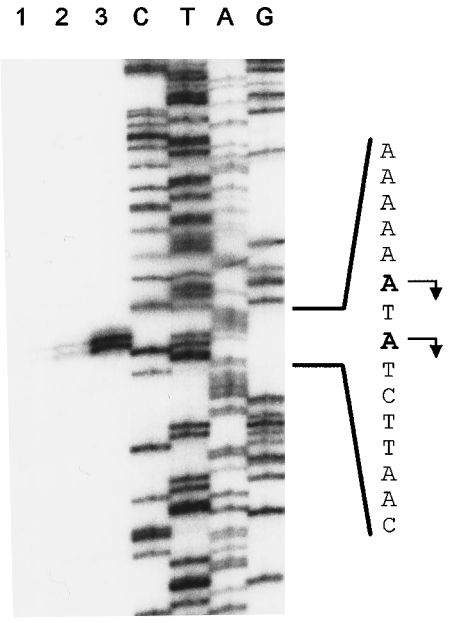

FIG. 3. Mapping of the start sites for the transcription of the *dra-nupC-pdp* operon. Total RNA was isolated from strains 168 and HH232 (*deoR*) and used in a primer extension analysis as described in Materials and Methods. RNA (20 μg) from wild-type strain 168 grown in the absence (lane 1) or in the presence (lane 2) of thymidine and from the *deoR* strain HH232 grown in the absence of thymidine (lane 3) was incubated with reverse transcriptase, deoxynucleoside triphosphates, and a single-stranded ^{32}P-labelled DNA primer complementary to nucleotides 1669 to 1684 in Fig. 2. The cDNA products were separated on a polyacrylamide gel. The same primer was used to create the sequence ladder (C-T-A-G).

TABLE 5. β-Galactosidase activity of wild-type and *deoR B. subtilis* strains carrying a *dra-lacZ* fusion integrated into the *amyE* locus[a]

Strain	Relevant genotype	Inducer added[b]	β-Galactosidase activity (nmol/min/mg)
HH219	*amyE*::*dra-lacZ*	None	10
		TdR	187 (18.7)[c]
		AdR	75 (7.5)
HH236	*amyE*::*dra-lacZ* *deoR*::*erm*	None	328

[a] Cells were grown in succinate minimal medium. Inducers were added to a final concentration of 1 mg/ml.
[b] TdR, thymidine; AdR, deoxyadenosine.
[c] Numbers in parentheses indicate fold of regulation.

was used. Two start sites of transcription positioned at nucleotides 1440 and 1442 were detected. The same two start sites were detected with primer 1. They were located 29 and 31 nucleotides, respectively, upstream of the putative translational start signal of the *dra* reading frame. A significant increase in the number of transcripts from the *dra* promoter was observed in thymidine-induced wild-type cells compared with the case for uninduced cells.

The intracellular inducer. In contrast to a wild-type strain, the *pdp* mutant LA804 exhibits no induction when thymidine is added to the medium, while the addition of deoxyadenosine still induces expression of both *dra* and the *pdp-lacZ* fusion (Table 3). The same induction pattern has been observed with various *pdp* mutants (data not shown). Therefore, thymidine is not the true internal inducer of the *dra-nupC-pdp* operon but a metabolite formed by the degradation of thymidine initiated by *pdp*. The immediate product of this reaction is dRib-1-P; this compound is likewise formed from deoxyadenosine by the action of purine nucleoside phosphorylase, which is present in all strains used for the presented induction studies. In *E. coli*, the inducer of the *deo* operon is formed when the enzyme phosphopentomutase converts dRib-1-P to dRib-5-P; therefore, mutants in the corresponding gene *deoB* are not inducible by thymidine. We performed the thymidine induction experiment with several independent *drm* mutants in *B. subtilis*, but in contrast to the case for *E. coli* they were all inducible by thymidine (data not shown). Rumyantseva and coworkers made the same observations (22). dRib-1-P, therefore, seems to act as the internal inducer in *B. subtilis.*

Like LA804 (*pdp*), the polar mutant HH221, showing no expression of *pdp*, exhibits no induction after thymidine is added (Table 3). In contrast, the polar mutant LA878, showing a low level of expression of *pdp*, does exhibit induction with thymidine. The low basal level of *pdp* expression in LA878 most likely originates from a promoter located in the integrated plasmid (a different plasmid was used for the construction of HH221).

Characterization of a *deoR* mutant. The open reading frame upstream of the *dra* gene was tentatively named *deoR*, since the 30% identity to the SorC repressor from *K. pneumoniae* indicated a regulatory function. In order to test the function of the DeoR protein, the *deoR* gene was disrupted by insertion of an *erm* antibiotic resistance cassette into the unique *Sma*I (Table 1). The direction of transcription was opposite to that of the *deoR* gene in the resulting strain HH232; thus, no transcription extends from the *erm* gene into the downstream genes, i.e., *dra*, *nupC*, and *pdp*. The levels of deoxyriboaldolase and thymidine phosphorylase were therefore measured in HH232, and as shown in Table 5, the *deoR* mutant had 10- to 20-fold-increased levels of these two enzymes when compared with the wild type (Table 2 or 3). Furthermore, they were no longer inducible. These results indicate that *deoR* encodes a negative regulator of expression of the *dra* promoter. To demonstrate more clearly the effect of the *deoR* mutation, a fusion of the *dra* promoter to *lacZ* was inserted into the *amyE* gene on the chromosome by using the plasmid pHH1014 (Fig. 1) containing the promoter which was located within the *Pvu*II-*Hin*cII fragment of plasmid pHH1011. The resulting strain HH219 exhibited 19-fold induction when thymidine was added, i.e., the *dra* promoter was intact and showed about 2-fold-better regulation when inserted in the *amyE* locus than when inserted in the native operon (Table 5). The *deoR*::*erm* mutation was introduced into HH219 in order to investigate the phenotype of the resulting strain HH236 when the *dra* promoter was located at the *amyE* locus in a true *trans* position to *deoR*. Strain HH236 showed very high levels of β-galactosidase, 33-fold above those of the uninduced wild-type HH219 (Table 5). The *deoR*::*erm* mutation was also introduced into strain HH218 (*deoR-dra-lacZ*::*amyE*), which contains the 1.9-kb *Eco*RI-*Sph*I fragment covering the *deoR* gene and the *dra* promoter region inserted in the *amyE* locus, resulting in strain HH235 (*deoR*::*erm deoR-dra-lacZ*::*amyE*). HH235 was grown in succinate minimal medium, and the pyrimidine nucleoside phosphorylase activity was 31 U/mg of protein without thymidine added and 250 U/mg of protein in the presence of thymidine. These results strengthen our suggestion that the *deoR* gene product functions as a *trans*-acting repressor. RNA isolated from cells of strain HH232 grown in the absence of thymidine was used in a primer extension experiment similar to the one described for the wild type (Fig. 3). Judging from the band intensities on the autoradiogram, the number of transcripts in the uninduced *deoR* strain is severalfold higher compared with that for both uninduced and induced wild-type cells. The *deoR-lacZ* transcriptional fusion harbored in HH230 (Table 3) was constructed to test if expression of *deoR* was inducible by thymidine. However, even in the presence of the inducer, the β-galactosidase levels were not above the background level found in strains not containing *lacZ* (e.g., HH221 [Table 3]).

DISCUSSION

Insertional inactivation of the *B. subtilis pdp* gene resulted in a mutant devoid of both thymidine and uridine phosphorylase activity (Table 3), confirming previous results demonstrating that only one enzyme, pyrimidine nucleoside phosphorylase, showing activity towards both thymidine and uridine, exists in *B. subtilis* (22). The predicted amino acid sequence of the Pdp protein shows 42% identity with that found for the thymidine phosphorylase (DeoA) isolated from *E. coli*. The regions from amino acid 82 to 90 (Val-Asp-Lys-His-Ser-Thr-Gly-Gly-Val) and from 113 to 123 (Ser-Gly-Arg-Gly-Leu-Gly-His-Thr-Gly-Gly-Thr) in the *E. coli* DeoA protein, which have been proposed to participate in the binding of phosphate and thymidine, respectively (31), are 100% conserved. In addition, the residues Arg-171, Ser-186, and Lys-190 in the DeoA protein, also most likely involved in thymidine binding, are preserved in the Pdp protein (residues 169, 184, and 188). With respect to the ability to cleave both pyrimidine ribonucleosides and pyrimidine deoxyribonucleosides, the pyrimidine phosphorylase from *B. subtilis* resembles uridine phosphorylase from *E. coli* (9). No homology, however, between the predicted amino acid sequences of the Pdp and Udp proteins was observed.

The amino acid sequence derived from the gene upstream of *pdp* was found to be 55% identical to the sequence of the NupC nucleoside transport protein from *E. coli*, and the corresponding gene from *B. subtilis* was therefore also named

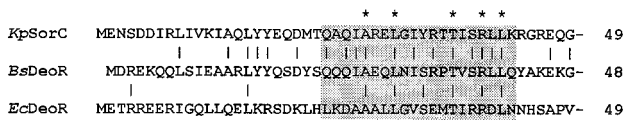

FIG. 4. Comparison of the putative DNA-binding regions of the DeoR proteins of *E. coli* and *B. subtilis* and of the SorC protein of *K. pneumoniae*. The sequence of *Bs*DeoR, *B. subtilis* deoxyribonucleoside repressor, was taken from Fig. 2. *Kp*SorC, *K. pneumoniae* sorbitol repressor (GenBank accession no. X66059 [unpublished]). *Ec*DeoR, *E. coli* deoxyribonucleoside repressor (GenBank accession no. X02837 [29]). The sequences for *K. pneumoniae* SorC and *E. coli* DeoR were obtained from GenBank. Identity between the *B. subtilis* sequence and the sequences of *K. pneumoniae* and *E. coli* is indicated by vertical lines. The shaded box indicates the position of putative DNA-binding sites. Asterisks above the *K. pneumoniae* sequence indicate amino acids identical in all three sequences.

nupC. The phenotype of the *nupC*::pLNA138 mutant (carrying a *nupC* disruption) showed reduced uridine transport, while inosine transport was similar to that in the parent strain and in the *pdp* control strain (Table 4). The NupC protein, therefore, seems to be the main system responsible for the transport of uridine and, possibly, pyrimidine nucleosides in *B. subtilis*, and it may thus be identical to the (deoxy)uridine/(deoxy)cytidine uptake system characterized by Kloudova and Fucik (12). In contrast, two nucleoside transport systems in *E. coli* have been characterized, and both of these systems (NupC and NupG) transport pyrimidine nucleosides efficiently.

The open reading frame found upstream of *nupC* showed 48% identity to the *deoC*-encoded deoxyriboaldolase of *M. pneumoniae*. The corresponding gene, named *dra*, in *B. subtilis* has previously been mapped close to the *pdp* gene (22).

Finally, the open reading frame upstream of *dra* showed 30% identity to the SorC repressor from *K. pneumoniae* (Fig. 4), thus indicating a function in gene regulation. We named the gene *deoR* (like the *E. coli* gene), since in *B. subtilis* also the gene regulates expression of proteins involved in deoxyribonucleoside catabolism. According to our results, the regulation of the operon expression by DeoR occurs at the level of transcription initiation. Furthermore, our data suggest that dRib-1-P is the true inducer.

The *E. coli* genes corresponding to *dra* and *pdp* are *deoC* and *deoA*, respectively. They are part of the operon *deoP1-deoP2-deoCABD*. The *deoR* repressor regulates expression of both *deoP1* and *deoP2* and responds to all deoxyribonucleosides being cleaved by the cell, the true inducer being dRib-5-P. The two distal genes of the *deo* operon, *deoB* and *deoD*, encode phosphodeoxyribomutase and purine nucleoside phosphorylase, respectively. All four Deo enzymes are thus involved in degradation of deoxyribonucleosides. Hence, the regulation by the DeoR repressor, with dRib-5-P acting as inducer, ensures an appropriate response to deoxyribonucleosides in the medium. Also, nucleoside transport is induced in *E. coli*, since one of the uptake systems, the most universal one, NupG, is regulated by DeoR. The results presented in this paper demonstrate a DeoR-regulated operon, *dra-nupC-pdp*, involved in catabolism and transport of pyrimidine ribonucleosides and deoxyribonucleosides. Also, another enzyme is needed for this purpose: phosphodeoxyribomutase, converting dRib-1-P to dRib-5-P and encoded by *deoB* in *E. coli* and *drm* in *B. subtilis*. While *deoB* is part of the *deo* operon, *drm* is located far from *pdp* at 182° on the linkage map (22). Phosphodeoxyribomutase is inducible by deoxyribonucleosides in *B. subtilis*, but the levels were not increased by the *deoR* mutation (data not shown). The *drm* expression therefore seems to be modulated by regulatory factors other than *deoR*. No induction of expression from *dra*, *pdp*, or *drm* by ribonucleosides has been observed in *B. subtilis*, in spite of *pdp* and *drm* also being involved in the degradation of pyrimidine ribonucleosides. This also contrasts with the case for *E. coli*: cytidine and adenosine may serve as the true inducers for the CytR repressor acting on the *deoP2* promoter, which in the presence of cAMP-CRP is a very powerful promoter. This induction of the Deo operon and of both the NupC and the NupG transport systems ensures that ribonucleosides are used very efficiently as the carbon source in *E. coli*. Since no cAMP-CRP system is present in *B. subtilis*, it is possible that a CytR homolog is missing.

The expression of the *dra-nupC-pdp* operon is subjected to glucose repression (catabolite repression). Three components are believed to be involved in catabolite repression in *B. subtilis*: (i) a catabolite responsive element (CRE), (ii) the CcpA protein, and (iii) the Hpr protein. The CRE is a 14-bp palindromic sequence found both upstream and downstream of the transcriptional start site of the regulated genes (11). We have located an almost consensus-like CRE in the start of the *dra* reading frame (nucleotides 1505 to 1518). No experiments have yet been performed to prove whether this putative CRE sequence or the CcpA and Hpr proteins are involved in catabolite repression of the operon.

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