The **yexA** gene product is required for phosphoribosylformylglycinamidine synthetase activity in *Bacillus subtilis*

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**INTRODUCTION**

The formation of inosine monophosphate (IMP) from 5′-phosphoribosyl 1′-pyrophosphate (PRPP) proceeds through an 11-step enzyme-catalysed pathway known as the *de novo* purine biosynthetic pathway. The fourth step in biosynthesis of purines is catalysed by the enzyme phosphoribosylformylglycinamidine (FGAM) synthetase (EC 6.3.5.3), which catalyses the reaction 5′-phosphoribosylformylglycinamide (FGAR) + glutamine + ATP → FGAM + glutamate + ADP + P\(_i\). Functional FGAM synthetases purified from chicken liver, *Salmonella typhimurium* and *Escherichia coli* consist of monomeric polypeptides of 133 kDa, 135 kDa and 141 kDa, respectively (Schendel et al., 1989). Antibodies raised against FGAM synthetase from Chinese hamster ovary cells cross-react with a human protein with a molecular mass of 150 kDa (Barnes et al., 1994).

The *E. coli* FGAM synthetase is encoded by the *purL* gene. The PurL polypeptide contains two domains, an N-terminal ATPase domain and a C-terminal glutamine-binding domain (Schendel et al., 1989). Nucleotide sequence analysis of the *pur* operon of *Bacillus subtilis* indicated that FGAM synthetase in this organism is encoded by two genes, *purQ* and *purL* (Ebbole & Zalkin, 1987). Alignment of the primary structure of the *E. coli* FGAM synthetase and the structure of the *B. subtilis* Q and L subunits showed that the PurQ sequence is homologous to the C-terminal part and the *purL* sequence is homologous to the N-terminal part of the *E. coli* PurL sequence. This indicated that the N- and C-terminal domains of FGAM synthetase in *B. subtilis* are located on separate proteins.

The *B. subtilis* *pur* operon has the gene order *purE*, *K*, *B*, *C*, *yexA*, *Q*, *L*, *F*, *M*, *N*, *H* and *D* (Ebbole & Zalkin, 1987). The different genes are organized in groups with overlapping coding regions: *purE–K–B*; *purC–yexA–purQ–L–F*; *purM–N–H*; and *purD*. The open reading frame designated yexA located immediately upstream of *purQ* encodes an unknown function. *yexA* has a reading frame that overlaps with both *purC* and *purQ*. In this report we present evidence that FGAM synthetase in *B. subtilis* is encoded by the *yexA* gene.
**Table 1.** Bacterial strains, plasmids and PCR primers

<table>
<thead>
<tr>
<th>Strain/plasmid/PCR primer</th>
<th>Characteristics</th>
<th>Reference, source or coordinates*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B. subtilis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>168</td>
<td>trpC2</td>
<td>C. Anagnostopoulos, CNRS, Jouy-en-Josas, France</td>
</tr>
<tr>
<td>BFA2257</td>
<td>trpC2 yexA::pMutin1 (erm)</td>
<td>Transformation of 168 by pHH1053, ErR</td>
</tr>
<tr>
<td>HH306</td>
<td>trpC2 yexA::pHH1059 (cat)</td>
<td>Transformation of 168 by pHH1059, CmR</td>
</tr>
<tr>
<td>HH309</td>
<td>trpC2 yexA6</td>
<td>HH306 screened for CmR</td>
</tr>
<tr>
<td>HH330</td>
<td>trpC2 yexA6 amyE::pDG268 (neo)</td>
<td>Transformation of HH309 by pDG268neo digested with KpnI, NeoR</td>
</tr>
<tr>
<td>HH332</td>
<td>trpC2 yexA6 amyE::pDG268 (neo)::pHH1091 (yexA+) (erm)</td>
<td>Transformation of HH330 by pHH1091, ErR</td>
</tr>
<tr>
<td>ED148</td>
<td>trpC2 purM</td>
<td>CU869 (Saxild &amp; Nygaard, 1988)</td>
</tr>
<tr>
<td>ED249</td>
<td>trpC2 hisA1 thr-5 purL1</td>
<td>Saxild &amp; Nygaard (1988)</td>
</tr>
<tr>
<td><strong>E. coli</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MC1061</td>
<td>F- araD139 (ara-leu)7696 galE15 galK16 Δ(lac)X74 rpsL (StrR) hsdR2 (r− m−) mcrA mcrB</td>
<td>Laboratory stock</td>
</tr>
<tr>
<td><strong>Plasmids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pMutin1</td>
<td>ApR (E. coli), ErR (B. subtilis); integrational vector for knockout mutations and formation of transcriptional lacZ fusion; the IPTG-inducible Pspac promoter is introduced to ensure expression of downstream genes</td>
<td>Vagner et al. (1998)</td>
</tr>
<tr>
<td>pDG268neo</td>
<td>ApR (E. coli), NeoR (B. subtilis); vector used for integration of transcriptional lacZ fusions into the amyE gene of B. subtilis</td>
<td>Saxild et al. (1996)</td>
</tr>
<tr>
<td>pBOE335</td>
<td>ApR (E. coli) CmR (B. subtilis); integrational vector, pUC19 containing the cat gene cloned into the KasI site</td>
<td>Saxild et al. (1996)</td>
</tr>
<tr>
<td>pHH1053</td>
<td>pMutin1 digested with BamHI and HindIII ligated to a 156 bp PCR fragment (primers 07 + 08) digested with the same enzymes</td>
<td>This work</td>
</tr>
<tr>
<td>pHH1059</td>
<td>pBOE335 digested with EcoRI and PstI and ligated to a 399 bp PCR fragment (primers 01 + 02) digested with PstI and BamHI and to a PCR fragment (primers 03 + 04) digested with EcoRI and BamHI</td>
<td>This work</td>
</tr>
<tr>
<td>pHH1091</td>
<td>pMutin1 digested with HindIII and BamHI and ligated to a 1040 bp PCR fragment (primers 05 + 06) digested with the same enzymes; this cloning brings yexA expression under the control of the IPTG-inducible Pspac promoter</td>
<td>This work</td>
</tr>
<tr>
<td><strong>PCR primers†</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primer 01</td>
<td>5′-GCCCTTGCAAGAAGGCAGAGGCTTGAG-3′</td>
<td>701444–701460</td>
</tr>
<tr>
<td>Primer 02</td>
<td>5′-GCCGGAATTCCTTTATTATACATGATGATGAC-3′</td>
<td>701843–701821</td>
</tr>
<tr>
<td>Primer 03</td>
<td>5′-GCCGGAATTCCTTTATTATACATGATGATGAC-3′</td>
<td>702060–702077</td>
</tr>
<tr>
<td>Primer 04</td>
<td>5′-GCCGGAATTCCTTTATTATACATGATGATGAC-3′</td>
<td>702484–702468</td>
</tr>
<tr>
<td>Primer 05</td>
<td>5′-GCCGGAATTCCTTTATTATACATGATGATGAC-3′</td>
<td>701444–701460</td>
</tr>
<tr>
<td>Primer 06</td>
<td>5′-GCCGGAATTCCTTTATTATACATGATGATGAC-3′</td>
<td>702484–702468</td>
</tr>
<tr>
<td>Primer 07</td>
<td>5′-GCCGGAATTCCTTTATTATACATGATGATGAC-3′</td>
<td>701873–701891</td>
</tr>
<tr>
<td>Primer 08</td>
<td>5′-GCCGGAATTCCTTTATTATACATGATGATGAC-3′</td>
<td>702029–702013</td>
</tr>
</tbody>
</table>

* Coordinates were taken from the B. subtilis genome sequence (Kunst et al., 1997).
† Underlined sequence indicates the position of the restriction site.

**METHODS**

**Bacterial strains, growth media and growth conditions.** Bacterial strains and plasmids used in this work are listed in

subtilis (and most likely also in several other organisms), in addition to the PurQ and PurL subunits, also requires the presence of the yexA gene product for activity.
**Fig. 1.** Creation of the *yexA*6 in-frame deletion mutation and the strategy used to recombine the mutation into the *pur* locus of the *B. subtilis* chromosome. The relevant part of the *pur* operon is shown. The genes are not drawn to scale. Symbols: small numbered horizontal arrows indicate the position and orientation of the DNA primers used in the PCR reactions; B, *Bam*HI; P, *Pst*I; E, *Eco*RI; *P pur*, *pur* operon promoter; T, transcription terminator; *purC*, phosphoribosylaminomimidazole-succinocarboxamide synthetase; *purQ*, FGAM synthetase subunit; *bla*, β-lactamase; *cat*, chloramphenicol acetyltransferase.

Table 1. As defined medium for *B. subtilis* strains, Spizizen’s-salts buffered minimal medium (Saxild & Nygaard, 1987) supplemented with 40 mg l-tryptophan l-1, 1 mg thiamin. HCl l-1, 0.2% l-glutamate and 0.4% glucose was used. Where indicated, MOPS-buffered minimal medium was used (Saxild & Nygaard, 1991). L-broth was used as rich medium. Bacterial strains were cultivated at 37 °C and growth was measured by determining the OD%&!

Enzyme assays and labelling of phosphorylated intermediates of the purine biosynthetic pathway. Preparation of cell-free extracts and measurement of the activities of adenylosuccinate (sAMP) lyase (Saxild & Nygaard, 1991), phosphoribosylglycinamidase (GAR) synthetase (Houlberg *et al.*, 1983) and β-galactosidase (Miller, 1972) were carried out as previously described. Activity of FGAM synthetase using 14C-labelled FGAR was measured essentially as described by Houlberg *et al.* (1983). Protein was determined by the Lowry method. The intracellular accumulation of intermediates of the purine biosynthetic pathway in purine-starved cells was determined as described before (Saxild & Nygaard, 1988). Cells were grown in the presence of 0.1 mM xanthine. At OD%&! 0.5, cells were collected by filtration and resuspended in purine-free medium containing [32P]orthophosphate. 32P-labelled compounds from cells grown for 2 h in 1 mM phosphate MOPS-buffered minimal medium containing 0.4 MBq [32P]orthophosphate ml-1 were extracted with formic acid and separated on polyethyleneimine-impregnated cellulose on plastic sheets as described by Jensen *et al.* (1979).

DNA manipulations. Isolation of chromosomal DNA (Saxild & Nygaard, 1987) and plasmid DNA (Birnboim & Doly, 1972), and transformation of *B. subtilis* (Boylan *et al.*, 1972) and *E. coli* (Hanahan, 1983), were carried out by standard methods. Restriction enzyme digestions and alkaline phos-
... phatase treatment were done as described for the supplier (Gibco-BRL). For Southern blotting, DNA was blotted onto a nitrocellulose membrane (GeneScreen, NEN Research Products). The chemiluminescence labelling and detection kit Southern Light (Tropix, PE Applied Biosystems) was used for light detection of the hybridization products. The recipe supplied by the manufacturer was followed. PCR was done by incubating template DNA with forward and reverse primers (8 µg ml⁻¹ each), 0.5 mM dNTPs and 1 unit Taq polymerase (Amersham Pharmacia Biotech) in 10 mM Tris pH 7.0 and 0.5 mM Mg²⁺. The reactions were run in a Biometra Trio 48 Thermocycler for 30 cycles consisting of 1 min at 94 °C, 1 min at 55 °C and 1 min at 72 °C. The reaction product was purified by centrifugation through a Pharmacia GFX Spin column.

DNA sequencing. Sequencing of double-stranded DNA templates was performed by using the Amersham thermocycle chain-termination sequencing kit containing β-dideoxynucleotides (Amersham Pharmacia Biotech). Reaction products were separated on a 6% polyacrylamide gel and visualized by autoradiography.

Construction of an in-frame deletion mutation in yexA. The method used to construct strain HH309 (yexA6) is illustrated in Fig. 1. Two fragments containing the 5' and the 3' end of the yexA reading frame, respectively, were generated by PCR. The fragment containing the 5' end was digested with PstI and BamHI and the other fragment was digested with EcoRI and BamHI. The fragments were simultaneously ligated into plasmid pBOE335 digested with PstI and EcoRI. The resulting plasmid, pHH1059, was transformed into B. subtilis strain 168 selecting for Cm². Since pBOE335 cannot replicate in B. subtilis the plasmid integrates into the chromosome in the pur locus as illustrated in Fig. 1. The resulting strain, HH306, was grown for several generations in the absence of Cm and plated on L-broth agar plates without Cm. The resulting colonies were then tested on Cm-containing agar plates and Cm² clones were isolated. One of the clones (HH309) was chosen, and primers 01 and 04 were used to generate a PCR fragment that covers the yexA region. Sequence analysis of the PCR fragment revealed that the replacement of the wild-type yexA allele with the yexA6 in-frame deletion was successful.

Cloning and expression of yexA from an IPTG-inducible promoter. Primers 05 and 06 (Table 1) were used to generate a 1040 bp PCR fragment containing the 3' end of the purC gene, the entire yexA reading frame and the 5' end of the purQ gene. The fragment was ligated into plasmid pMutin1. The resulting plasmid, pHH1091, contains the yexA gene placed after the IPTG-inducible Pspac promoter and in front of the lacZ reporter gene (Vagner et al., 1998) as illustrated in Fig. 2. Plasmid pHH1091 was transformed into strain HH330, which contains the plasmid pDG268neo integrated into the amyE gene. Since no promoter is present in front of the lacZ gene of pGD268, strain HH330 is β-galactosidase-negative. Because pHH1091 cannot replicate in B. subtilis, Er² transformants of strain HH330 will only be formed if recombination between the two identical lacZ sequences or the two identical yexA sequences occurs. Five Er² transformants were analysed by Southern blotting and one clone (HH332, Fig. 2) with pHH1091 recombined into the amyE locus was isolated.

RESULTS
Integration of pMutin1 into the yexA locus and phenotype of a yexA mutant strain
A 156 bp PCR product internal to the yexA reading frame was amplified by the use of primers 07 and 08 (Table 1). The DNA fragment was digested with HindIII and BamHI and cloned into the HindIII and BamHI sites of plasmid pMutin1, resulting in pHH1053. Derivatives of plasmid pMutin1 contain – in addition to a part of the gene to be knocked out, here yexA – the Pspac promoter under the control of the lacI gene product. The

![Fig. 2. Recombination of a Pspac-yexA⁺ construction into the lacZ gene located in the amyE locus in strain HH330 (amyE::pDG268neo yexA6). Symbols: white-headed black arrows indicate plasmid-encoded genes; thin arrows indicate the direction of transcription of the gene in question; erm, erythromycin-resistance gene; neo, neomycin-resistance gene; lacZ, β-galactosidase; amyE, α-amylase (5' part); amyEb, α-amylase (3' part): lacI, E. coli lactose operon repressor. The rest of the symbols are defined in the legend to Fig. 1.](Image 130x526 to 486x734)
promoter drives the transcription of the region down- 
stream of the integration point and can be induced by 
IPTG (Vagner et al., 1998). Plasmid pH1053 was 
integrated into the yexA gene by a single homologous 
recombination event, which was confirmed by Southern 
blot analysis (data not presented). When tested for 
growth on minimal agar plates the resulting strain 
(BFA2257) had a purine-auxotrophic phenotype both 
with and without added IPTG. BFA2257 could use 
hypoxanthine as purine source, which indicates that the 
purine-negative phenotype was due to a defect in the 
formation of IMP (Saxild & Nygaard, 1988).

**Isolation and phenotype of a mutant strain 
containing an in-frame deletion in yexA**

The five genes purC, yexA, purQ, purL and purF have 
overlapping coding sequences, which might indicate 
translational coupling (Ebbole & Zalkin, 1987). Trans- 
lational coupling means that translation of a certain 
gene requires the translation of the overlapping up- 
stream gene. In *B. subtilis* translational coupling has 
been shown to be important for the expression of the 
*pbuX* gene (Christiansen et al., 1997), but has not been 
demonstrated to function in the expression of genes in the 
*pur* operon (Ebbole & Zalkin, 1989). However, it 
could not be excluded that the plasmid integration into 
yexA in BFA2257 affects the translation of the down- 
stream gene *purQ* and that the purine-negative phenotype 
was due to *purQ* deficiency and not caused by the 
disruption of yexA. To test this possibility, an in-frame 
deletion of 73 codons of the yexA reading frame was 
constructed and recombined into the chromosome as 
described in Methods (Fig. 1). The resulting strain, 
HH309, also showed a purine-negative phenotype. Like 
BFA2257, HH309 was able to use hypoxanthine as a 
purine source, indicating a similar defect in both mutant 
strains.

**Identification of the metabolic block in the yexA 
mutants**

To narrow down the biochemical defect in the yexA 
mutants we determined their purine enzyme levels, 
growth on different purine sources, thiamin require- 
ment, and accumulation of intermediary compounds 
of the purine biosynthetic pathway under conditions 
of purine starvation as described before (Saxild & 
Nygaard, 1988).

The accumulation studies showed an accumulation of 
FGAR in the yexA mutant, which indicates a block in the 
reaction catalysed by FGAM synthetase. The key 
experiment is shown in Fig. 3. Strain ED249 (purL) 
accumulated FGAR and ED148 (purM) accumulated 
both FGAR and FGAM. Strain HH309 (yexA6) ac- 
cumulated large amounts of FGAR, thus resembling the 
purL strain ED249. The phenotypic similarity of HH309 
To ED249 indicates that FGAM synthetase activity in 
HH309 is impaired. Strain BFA2257 was subjected to 
purine starvation in both the presence and absence of 
IPTG. As mentioned above, IPTG induces the *P*<sub>space</sub> 
operator, which results in the transcription of genes 
located downstream of the pMutin1 insertion point 
(Vagner et al., 1998). In the *B. subtilis* pur operon the 
purQ, L, F, M, N, H and D genes are located downstream of yexA (Ebbole & Zalkin, 1987). When 
BFA2257 was starved for purine in the absence of IPTG 
a very low amount of FGAR was produced. Purine 
starvation in the presence of IPTG resulted in the 
accumulation of large amounts of FGAR. So in the 
presence of IPTG BFA2257 is phenotypically FGAM 
synthetase negative. It can therefore be concluded that
Table 2. Level of purine biosynthetic enzymes in the wild-type and yexA mutant strains grown in the presence of different purine compounds

Cells were grown in glucose minimal medium supplemented with the stated purine compound (xanthine, 0.1 mM; adenine, 1 mM; guanosine, 1 mM) and with or without IPTG. Enzyme activities were determined as described in Methods. The listed values are means of three experiments.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Addition to medium</th>
<th>t_d* (min)</th>
<th>Enzyme activity [nmol min⁻¹ (mg protein)⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>β-Galactosidase (lacZ) sAMP lyase (purB) GAR synthetase (purD)</td>
</tr>
<tr>
<td>168</td>
<td>Wild-type</td>
<td>None</td>
<td>46</td>
<td>ND 221 13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Xanthine</td>
<td>48</td>
<td>1578 118 13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adenine + Guanosine</td>
<td>48</td>
<td>ND 117 18</td>
</tr>
<tr>
<td>BFA2257</td>
<td>yexA::pMutin1</td>
<td>Xanthine</td>
<td>52</td>
<td>1578 239 &lt;1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Xanthine + IPTG</td>
<td>50</td>
<td>550 194 7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adenine + Guanosine</td>
<td>48</td>
<td>55 16 &lt;1</td>
</tr>
<tr>
<td>HH309</td>
<td>yexA6</td>
<td>Xanthine</td>
<td>52</td>
<td>ND 221 42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adenine + Guanosine</td>
<td>48</td>
<td>ND 12 4</td>
</tr>
<tr>
<td>HH332</td>
<td>yexA6 Pspac−yexA*</td>
<td>Xanthine</td>
<td>52</td>
<td>152 235 49</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Xanthine + IPTG</td>
<td>48</td>
<td>125 117 49</td>
</tr>
</tbody>
</table>

ND, Not determined.
* t_d, doubling time.
† Only relevant genes are noted. The gene order of the pur operon is Ppur−E−K−B−C−yexA−Q−L−F−M−N−H−D−T. Arrows indicate the various transcripts formed in the cell under the growth condition in question. P, promoter; T, terminator.

The downstream-located purF, purD and purN genes, which encode the first three steps of the pathway (Fig. 3), are only weakly expressed in the absence of IPTG, but efficiently expressed in the presence of IPTG. If these genes are expressed, purQ and purL are most likely also expressed. However, it could not be exclusively concluded that the accumulation of FGAR in strain HH309 and in BFA2257 grown in the presence of IPTG was due to the lack of the yexA gene product. The possibility that the mutational changes could result in some kind of polar effect on the expression of the downstream-located purQ and purL genes could not be ruled out.

Effects of the yexA mutation on the expression of the pur operon

The levels of sAMP lyase, encoded by the purB gene located upstream of yexA, and of GAR synthetase, encoded by the purD gene located downstream of yexA, were determined in wild-type strain 168 and the yexA mutant strains BFA2257 and HH332. Two growth conditions were used. In one, xanthine served as the purine source. In this condition purine gene expression is neither derepressed nor repressed. In the other condition adenine and guanosine were added as purine source. In this condition the purine gene expression is maximally repressed (Saxild & Nygaard, 1991). As expected, enzyme levels in the wild-type were not repressed by xanthine, whereas addition of adenine plus guanosine resulted in reduced enzyme levels (Table 2).

In both mutant strains (BFA2257 and HH309) reduced growth rate in the xanthine medium and increased levels of sAMP lyase were observed. In strain BFA2257 β-galactosidase activity driven by the pur promoter was assessed and shown to follow the same pattern of expression as purB. The increased sAMP lyase level indicates that BFA2257 suffers from a partial purine starvation. The lack of GAR synthetase activity indicates that transcription of the purD gene, which is located downstream of the pMutin1 insertion point in yexA, was markedly reduced. However, if IPTG was present, significant expression of purD was observed. This indicates that genes located downstream of yexA were transcribed from the Pspac promoter in the presence of IPTG. In strain HH309 the expression of both purB and purD genes responds as in wild-type cells to the addition of either xanthine or a mixture of adenine and guanosine, indicating normal transcriptional control of genes located downstream of yexA. The possibility could not be excluded that translation of the purQ gene located downstream of yexA, and which has overlapping coding sequence with yexA, was affected by the pMutin insertion. However, strain HH332, which harbours the yexA6 allele in-frame deletion, was able to express the purD gene. This suggests normal transcription of genes located downstream of yexA. Because the reading frame of yexA6 is continuous and identical to the wild-type reading frame one would expect normal expression of the overlapping downstream purQ gene. Most likely both the purQ and purL genes, which encode subunits of
Table 3. Level of FGAM synthetase activity in wild-type and yexA mutant strains as affected by PurQ, PurL and YexA

<table>
<thead>
<tr>
<th>Extract no.</th>
<th>Subunit contents of the resulting extract</th>
<th>FGAM synthetase activity (nmol min⁻¹ mg⁻¹)</th>
<th>Source strain</th>
<th>Relevant genotype</th>
<th>Addition to growth medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PurQ PurL YexA</td>
<td>3.8</td>
<td>168</td>
<td>Wild-type</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>PurQ PurL</td>
<td>&lt;0.05</td>
<td>HH309</td>
<td>yexA6</td>
<td>Xanthine</td>
</tr>
<tr>
<td>3</td>
<td>PurQ PurL YexA</td>
<td>1.6</td>
<td>HH332</td>
<td>yexA6 Pspac-yexA⁺</td>
<td>IPTG</td>
</tr>
<tr>
<td>4</td>
<td>YexA</td>
<td>&lt;0.05</td>
<td>HH332</td>
<td>yexA6 Pspac-yexA⁺</td>
<td>Adenine + Guanosine + IPTG</td>
</tr>
<tr>
<td>2 + 4*</td>
<td>PurQ PurL YexA</td>
<td>1.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Equal amounts of cell extracts were present in the assay mixture.

the FGAM synthetase enzyme, are expressed and what is lacking for FGAM synthetase activity is the yexA gene product.

Complementation analysis

To see if the yexA gene product could be supplemented in trans the yexA gene was inserted in another location on the chromosome. Expression of yexA was put under the control of the Pspac/LacI system by ligation of a PCR fragment containing yexA (Table 1) into plasmid pMutin1. The resulting plasmid, pHH1091, was transformed into strain HH330 [yexA6 amyE::pDG268neo] as described in Methods. The resulting strain HH332 [yexA6 amyE::pDG268neo::pHH1091 (yexA⁺)] (Fig. 2) required either IPTG or purine for growth and expressed both yexA and lacZ from the Pspac promoter. When cells were grown in minimal medium, β-galactosidase activity was increased when IPTG was added (Table 2). The lacZ expression reflects the expression of yexA in trans, which is built into the construction in front of the lacZ gene (see Fig. 2). It therefore was evident that YexA, when produced in trans, could complement the yexA6 deletion mutation.

Whether the YexA protein was required for FGAM synthetase activity in vitro was assessed in wild-type cells, in the yexA mutant strain HH309 and in strain HH332, in which the level of PurQ, PurL and YexA could be manipulated (Table 3). FGAM synthetase activity was found in strain 168, but not in strain HH309, which only produces the PurQ and the PurL protein. However, FGAM synthetase activity was found in strain HH322 when grown in the presence of IPTG. This was expected because of the way the construction was made. When adenine and guanosine were added together with IPTG to the growth medium no FGAM synthetase activity was found. Under these conditions the adenine and guanosine repress the expression of the pur operon and therefore also the synthesis of PurQ and PurL. However, when extracts of cells synthesizing PurQ and PurL (extract 2, Table 3) and extracts of cells synthesizing YexA (extract 4, Table 3) were combined, FGAM synthetase activity was restored. These results prove that the yexA gene product is required for FGAM synthetase activity in B. subtilis and that yexA therefore must be considered as a novel purine biosynthetic gene. We suggest that the yexA gene is given the designation purS.

DISCUSSION

Since 1996 two research consortiums – a European and a Japanese – have performed systematic gene function analysis in B. subtilis (Vagner et al., 1998). One of the obligations for each of the research groups involved has been, in an assigned genomic region, to systematically inactivate all genes for which no obvious function could be suggested from the deduced amino acid sequence. The authors of this report were assigned the region sequenced by Borriss et al. (1996), which includes the pur operon. The function for 11 of the 12 genes of the pur operon has been identified from sequence similarity studies (Ebbol & Zalkin, 1987) or by experimental means (Saxild & Nygaard, 1988; Saxild et al., 1994). Only the function of the yexA reading frame, which is located between purC and purQ, has remained unsolved. However, after publication of the nucleotide sequence, possible functions of yexA have been suggested. Ebbole & Zalkin (1987) showed that the expression of yexA in E. coli gave rise to a small protein and they suggested a regulatory function of this protein. More recently, Peltonen & Mantslää (1999) observed that reading frames similar to the yexA gene exist in other bacteria and they suggested that the yexA gene product was required for functional FGAM synthetase activity. However, they provided no experimental evidence to support this suggestion. In this report we demonstrate that a B. subtilis yexA mutant lacks FGAM synthetase activity and that it accumulates the substrate FGAR during purine starvation. We suggest that yexA is
renamed as purS. It appears that an extra peptide, PurS (YexA), is required for FGAM synthetase activity in bacteria like *B. subtilis* in which the enzyme is composed of heteromers. Since FGAM synthetase activity can be restored *in vitro* by adding extracts containing PurQ and PurL to an extract containing only PurS, we suggest that one possible role of PurS is to act as a hinge that links the two catalytic subunits. Another possibility is that PurS plays a role in catalysis. On the other hand we can exclude that PurS is required for stabilizing the PurQ and PurL subunits since we were able to synthesize biologically active PurQ and PurL proteins in the absence of PurS. yexA homologues most likely exist in cyanobacteria [*Synechocystis* sp. (accession no. Q55842)], methanogens [*Methanococcus jannaschii* (accession no. Q58988)] and *Methanobacterium thermoautotrophicum* (accession no. 026271)] and the Gram-positive bacteria *Mycoplastema tuberculosis* (accession no. AL123456), *Mycobacterium leprae* (accession no. 005755) and *Lactococcus lactis* (Peltonen & Mänttäri, 1999). These organisms all contain the multi-subunit form of FGAM synthetase encoded by purQ and purL genes that are similar to those found in *B. subtilis*.

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**REFERENCES**


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