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# Enhanced Expression of β-Xylosidase of *Bacillus stearothermophilus* No. 236 by Change of Translational Initiation Codon in *Escherichia coli* and *Bacillus subtilis*

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**Abstract** The *xylA* gene of *Bacillus stearothermophilus* No. 236 encoding β-xylosidase, a major xylanolytic enzyme, was previously cloned and sequenced by the present authors. Sequence analysis indicated that translation of the *xylA* gene was initiated from the noncanonical initiation codon UUG, confirmed by analyzing three different amber (UAG) mutants of the *xylA* gene. In the present study, the UUG initiation codon was mutated into AUG or GUG, and the effects of the mutations on the XylA synthesis were examined. The AUG initiation codon was found to direct the highest level of β-xylosidase synthesis; three-fold and fourteen-fold more enzyme activity than the UUG codon in *E. coli* and *B. subtilis* cells, respectively. Surprisingly, contrary to other systems reported to date, the UUG start codon was found next to AUG in the relative order of translational efficiency in both organisms. In addition, a greater abundance of the *xylA* mRNA was detected with the AUG start codon in both of these host cells than with GUG or UUG. Northern blot and Toeprint assays revealed that this was due to enhanced stability of mRNA with the AUG initiation codon. As expected, the β-xylosidase protein level in the bacterial cells containing mRNA with the AUG start codon was also much higher than the levels with the other two different mRNAs.

**Key words:** Translation initiation, β-xylosidase, *Bacillus stearothermophilus*

Protein synthesis by decoding the information in mRNAs is a complex, continuous process comprising initiation, elongation, and termination stages. Initiation is generally the rate-limiting step of translation and requires the placement of a start codon onto the ribosome P site. In prokaryotes as well as eukaryotes, AUG is known to be the most frequently used and also most efficient initiation codon, however, in bacteria, GUG and UUG also can frequently serve for initiation with the usage frequencies of around 8 and 1% in *E. coli*, respectively [9, 13, 19].

Besides the importance of the start codon for initiation, other translation factors and signals also contribute to the binding of mRNA to ribosomes. The Shine-Dalgarno (SD) sequence [15], which is located upstream of the initiation codon, is a highly characterized translation signal of prokaryotic mRNAs and is complementary to the anti-Shine-Dalgarno (ASD) sequence near the 3' end of the 16S rRNA [2, 12, 13]. The SD sequence requires a minimum distance to the initiation codon in order to drive translation, and both *E. coli* and *B. subtilis* exhibit similar spacing optima of 7–9 nucleotides [13, 19].

In our earlier study, a bacterium possessing a strong xylanolytic enzyme system was isolated from soil and identified as *Bacillus stearothermophilus* No. 236 [16]. This strain was capable of producing all the enzymes necessary for complete xylan biodegradation [3, 5, 7, 17, 18]. The genes encoding the xylanolytic enzymes, including the *xylA* gene which codes for β-xylosidase, a major xylanolytic enzyme, have been cloned and sequenced [8, 10, 11].

The *xylA* gene of *B. stearothermophilus* consists of an ORF of 1,830 bp directing 609 amino acids [10]. The initiation codon of the ORF was identified as the non-canonical initiation codon UUG, and the SD sequence (5'-AGGAGG-3') was found 8 bp upstream of the initiation codon (Fig. 2).

In this study, the UUG codon was converted to AUG or GUG by site-directed PCR mutagenesis, and the mutation effects on expression of the *xylA* gene in both *E. coli* and *B. subtilis* cells were examined. The results showed that the *xylA* transcripts with the AUG start codon bound to the ribosome more strongly than those with UUG or GUG start codons, resulting in a greater mRNA stability. The

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enhanced stability of the mRNA in turn resulted in higher level of β-xylosidase synthesis, especially in *B. subtilis*.

## MATERIALS AND METHODS

### Bacterial Strains and Plasmids

Host strains for various plasmids used in this work were *E. coli* XL1-Blue (*recA1 endA1 gyrA46 thi-1 hsdR17 supE44 relA1 lac* F' [*proAB lacI*$^{q}$ZΔM15 Tn10(Tet$^{r}$)]) (Stratagene), and *B. subtilis* strain MW15 (*his nprR2 nprE18 ΔaprA3 ΔeglS102 ΔbglT bglSRV ΔxynA* Cm$^{R}$). The plasmid pWPBR was used as an *E. coli* and *B. subtilis* shuttle vector (provided by S. S. Park, Korea University), and the recombinant plasmid pMG1 [8] was used as a source of the *xylA* gene of *Bacillus stearothermophilus* No. 236.

### Culture Conditions

The *E. coli* XL1-Blue and *B. subtilis* MW15 strains carrying pWPBR-1 were grown at 37°C in Luria-Bertani (LB) medium supplemented with 50 μg/ml of ampicillin and 10 μg/ml of kanamycin, respectively.

### Measurement of the β-Xylosidase Activity

β-Xylosidase activity was assayed by monitoring the liberation of *p*-nitrophenol from 10 mM *p*-nitrophenyl-β-D-xylopyranoside (PNPX) in 50 mM phosphate buffer (pH 7.0) [11]. One hundred microliters of the enzyme solution were incubated at 45°C for 10 or 15 min with the equal volume of the substrate, and the reaction was terminated by adding 1 ml of 0.4 M $Na_2CO_3$. The released *p*-nitrophenol was determined by reading absorbance at 405 nm. One unit of the enzyme activity was defined as the amount of enzyme required to release 1 μmole of *p*-nitrophenol per min.

### Primer Extension

Primer extension reactions were carried out using total RNAs isolated from the *B. stearothermophilus* No. 236 cells and the radiolabeled synthetic primer Ext (5'-GT-CGAAGCCGCCGCTTTGGCCGGGATAAC-3'). After incubation, the reaction mixtures were electrophoresed against the appropriate dideoxy sequencing reactions and visualized with autoradiography.

### Construction of pWPBR-1

The 2.8 kb DNA fragment obtained by digesting pMG1 with *Hin*dIII was cloned into pWPBR, which had previously been linearized with *Hin*dIII, and treated with calf intestine alkaline phosphatase (Roche BM).

### Mutagenesis of the Plasmid-Encoded *xylA* Gene

Initiation codon mutations were introduced into the *xylA* gene on the plasmid pMG1 by PCR using a QuickChange™ site-directed mutagenesis kit (Stratagene). All the mutants obtained were verified by DNA sequencing.

### Western Blot Analysis

In order to tag 6 His residues at the C-terminus of the *xylA* gene, two primers were designed, based on the nucleotide sequence of pMG1; primer HisF (5'-GGACGAGATCT-AAGCTTGGCCGAATGACC-3') corresponding to the N-terminal region, and primer HisR (5'-GACGGCTCGAGT-TTCTCATCCAGCCACAA-3') corresponding to the C-terminal region of the *xylA* gene. The 2.6 kb fragment obtained from the PCR was inserted into the pET-23b(+) that had been digested by *Bgl*II and *Xho*I. The resulting plasmid was named pXylA. Proteins synthesized from the cells containing the recombinant plasmid described above were separated by 8% SDS-PAGE, and Western blotting was performed using primary mouse anti-His monoclonal antibodies (Santa Cruz Biotechnology Inc., U.S.A.) and secondary peroxidase-conjugated goat anti-mouse IgG (Sigma). Detection was carried out using the ECL detection system (Amersham Phamacia Biotech Inc., U.S.A.).

### Northern Blot Analysis

RNA preparation and Northern blot analysis were carried out according to the standard methods of molecular biology [14]. Ten micrograms of the total RNAs prepared from the *E. coli* and *B. subtilis* strains were loaded onto 1% formaldehyde-agarose gel. Following electrophoresis, the gel was blotted onto Hybond N$^{+}$ nylon membrane (Amersham Pharmacia Biotech Inc., U.S.A.), and then hybridized with the $^{32}$P-labeled DNA probe, which was a 200 bp DNA fragment of the *xylA* gene obtained by PCR with primer NorF (5'-TACGATGGAATCGAT-3') and primer NorR (5'-AATTGCGC TAACGC-3').

### RNA Synthesis

Messenger RNAs used in toeprint assays were generated *in vitro* using T7 RNA polymerase [6]. Templates for T7 transcription were prepared by gel-purifying the *Bgl*II-*Pst*I restriction fragments of the His-tagged *xylA* gene containing T7 promoter. The transcription reaction mixture contained 15 mM DTT, 4 mM NTPs, 40 mM Tris-HCl (pH 7.9), 20 mM $MgCl_2$, 1 μg of template DNA, and 500 U of T7 RNA polymerase. mRNAs were synthesized for 1 h at 37°C, followed by treatment with phenol/chloroform/isopropanol (25:24:1). The RNA separated was precipitated with sodium acetate (pH 6) and isopropanol. The final RNA concentration was determined by absorbance at 260 nm (assuming 1 $OD_{260}$=40 μg ml$^{-1}$ and 330 g mol$^{-1}$ nucleotide$^{-1}$).

### *In Vitro* Translation

*In vitro* translation assays in the *E. coli* S30 extract system were carried out according to the manufacturer's protocol as follows: mRNA (200 ng) was incubated for 15 min at 25°C

in a 20 μl reaction mixture containing 1 μl of KM solution (2 M KCl, 10 mM $MgCl_2$), 1 ml of creatine phosphate (0.2 M), 4 μl of complete nonradioactive amino acid mixture (1 mM; Promega), and 14 ml of *E. coli* S30 extract (Promega). After incubation, EDTA (final concentration, 5 mM) was added to the translation mixture to stop the reaction, and additional $MgCl_2$ (final concentration, 5 mM) was added prior to performing the primer extension reaction [4, 20].

### Primer Extension Inhibition (Toeprint) Assays

Toeprint assays were performed by adding 10 μl of $^{32}$P-end labeled primer Ext (5'-GTCGAAGCCGCCGCTTTGGC-CGGGATAAC-3') to 20 μl of the translation reaction mixture, and then 0.1 volume of 3 M sodium acetate (pH 5.2) and 2.5 volumes of absolute ethanol were added to the above mixture. After incubation for 30 min at -20°C, RNAs and the primer were spun down by centrifugation at 12,000 rpm at 4°C. Dried pellets of the centrifuged material were suspended in 15 μl of hybridization buffer [50 mM KCl, 25 mM Tris-HCl (pH 8.3)]. Then, the hybridization mixture was heated at 70°C for 2 min and slowly cooled down by turning the heating block off. When reached to room temperature, a reverse transcription reaction mixture [1.5 μl of 0.1 M DTT, 5 μl of 2.5 mM dNTP, 6 μl of 5× M-MLV buffer, and 1.2 μl of M-NLV (Promega, U.S.A.)] was added to the hybridization mixture. Subsequently, the reaction mixture was incubated for 60 min at 42°C. By adding 1 μl of 0.5 M EDTA, the reaction was terminated. Residual RNAs were degraded by treatment with 1 μl of pancreatic RNase A. The dried pellets obtained after phenol extraction and subsequent ethanol precipitation were dissolved in a loading buffer [80% formamide, 10 mM EDTA (pH 8.0), and 1 mg/ml of bromophenol blue], and analyzed on 6% denaturing polyacrylamide sequencing gel. DNA fragments were prepared, labeled with α-[$^{35}$S]-dATP, and identified by autoradiography on X-ray film. Size markers used in the primer extension analysis were prepared from the same $^{32}$P-end-labeled primer and the same mRNA used for the toeprint assay.

## Results and Discussion

### Identification of the Transcription Initiation Site of *xylA*

The *xylA* gene of *Bacillus stearothermophilus* No. 236 encoding β-xylosidase was cloned and sequenced previously [10, 11]. The transcriptional start site of the *B. stearothermophilus* No. 236 *xylA* gene was identified by primer extension analysis to be the guanine (G) located at 24 bp upstream from the translational initiation codon, the same site previously determined for the *xylA* gene expressed in *E. coli* (Fig. 1). This identification suggests

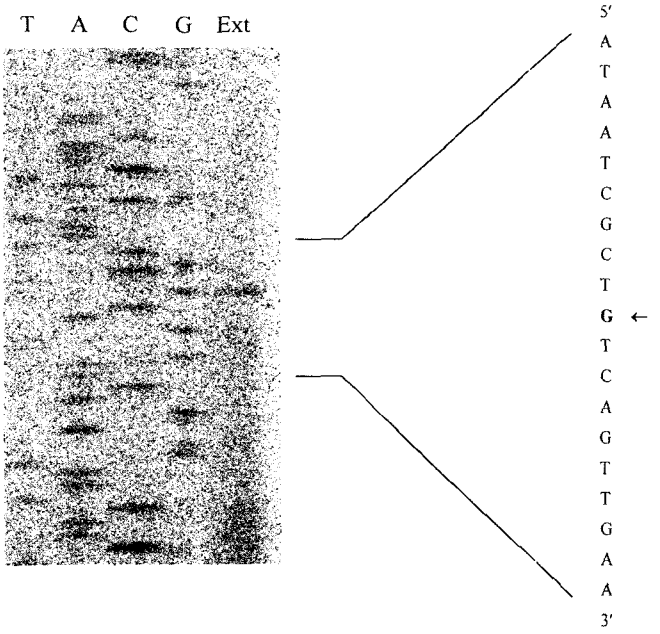


**Fig. 1.** Identification of the transcription start site of *xylA* by primer extension.
Primer extension reactions were carried out using total RNAs isolated from the *B. stearothermophilus* No. 236 cells and the radiolabeled synthetic primer Ext (5'-GTCGAAGCCGCCGCTTTGGCCGGGATAAC-3'). The cells were harvested when they reached the early mid-log phase, and total RNAs were prepared as described in Materials and Methods. The arrow indicates the transcription start site.

that the expression of *xylA* gene is directed in the *B. stearothermophilus* No. 236 cells by the previously determined transcription initiation signals: -10 sequence (CATAAT) and -35 sequence (TTGTTA) separated by 12 bp.

### Identification of the Translation Initiation Codon of *xylA*

Sequence analysis revealed that the *xylA* transcript was translated unusually from an unusual start cocon, UUG

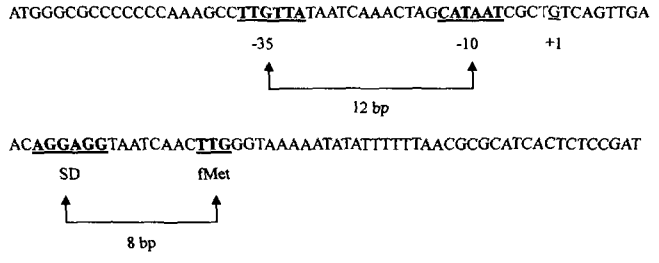


**Fig. 2.** Sequence of the β-xylosidase gene (*xylA*).
Based on the sequence analysis of the *xylA* gene [10], the putative -35 and -10 elements were determined to be TTGTTA and CATAAT, respectively. The hexametric DNA sequence elements were separated by 12 bp. The transcriptional start site of the *B. stearothermophilus* No. 236 *xylA* gene was identified as the guanine (+1). Translation initiation started from the TTG initiation codon. The putative Shine-Dalgarno sequence (5'-AGGAGG-3') was found 8 bp upstream of the initiation codon.

A

TGAACAGGAGGTAATCAACTTGGGTAAAAATATATTTTTTAACGCGCATCACTCTCC

SD −2 +1 +12

↓ ↓ ↓

amber stop codon amber stop codon

B

| Clone | Specific activity[a] (unit/mg) |
|---|---|
| *xylA* | 11.35 |
| -2AM | 13.87 |
| +1AM | 0.42 |
| +12AM | 0.61 |

**Fig. 3.** Identification of the translation initiation codon of *xylA* in *E. coli*.

(A) Replacement of the amber stop codons in *xylA*. To re-identify the real translation codon of *xylA*, amber stop codons were changed at selected sites within and outside the putative open reading frame (ORF) using a QuickChange™ Site-directed mutagenesis kit (Stratagene). The position of the initiation codon determined by this method is designed as +1; upstream codons are indicated by a minus sign. (B) β-Xylosidase activities of *xylA* with amber stop codon mutants. [a]One unit of enzyme was defined as the amount of enzyme required to release 1 μmole of *p*-nitrophenol per min. Enzyme activities (unit/ml) were determined from the intracellular fraction of the cultured *E. coli* cells. The values shown are the averages of two independent experiments.

(Fig. 2). To determine whether the predicted start codon actually served as a real translation initiation site of the *xylA* gene, three sites selected were changed to the amber stop codon (UAG) by site-directed mutagenesis within and outside the putative open reading frame (ORF) (Fig. 3A) [1]. The recombinant plasmids carrying each of these mutant *xyl*A genes (AM) were transformed into the *E. coli* XL1-Blue cells, and β-xylosidase activities were then assayed as described in Materials and Methods. The position of the putative initiation codon UUG is designated as +1, and upstream and downstream in-frame codons are indicated by minus and plus signs, respectively. If the previously proposed UUG codon actually functioned as a translation start codon, conversion of the UUG or any of the internal codons into the amber codon should not result in β-xylosidase activity. In contrast, mutation of upstream codons into the amber codon would have no effect on the expression of the *xylA* gene. As shown in Fig. 3B, the -2AM mutant produced β-xylosidase of nearly the same activity level as that for the wild-type *xylA* gene, while the +1AM and +12AM mutants showed no detectable activity. These results clearly indicated that translation of the *xylA* gene begins at the noncanonical initiation codon, UUG.

### Effect of the Translation Initiation Codon Mutants on the Translational Efficiency of *xylA*

The Shine-Dalgarno sequence requires a minimum distance to the initiation codon before translation can occur, and both *B. subtilis* and *E. coli* exhibit similar spacing optima of 7~9 nucleotides [13, 19]. As shown in Fig. 2, in the case of the *xylA* gene, the spacing is 8 nucleotides long which is considered optimal for translation in both organisms. Also, the translation initiation codon is another important determinant of ribosome binding strength and translational efficiency for mRNA; AUG is not only the most frequently occurring but also the most efficient initiation codon in all living organisms. However, as described above, the translation start codon of the *xyl*A gene is the noncanonical UUG, which is not the most preferred initiation codon. Therefore, in this study, UUG was converted into AUG or GUG by site-directed mutagenesis, and the 2.8 kb DNA fragments containing the complete *xylA* gene with the changed initiation codons were inserted into the *B. subtilis-E. coli* shuttle vector pWPBR. The recombinant plasmids obtained were then transformed into the *E. coli* XL1-Blue and *B. subtilis* MW15 cells, and β-xylosidase activity was measured. As presented in Table 1, the AUG initiation codon directed the product synthesis about 3-fold more than the UUG in *E. coli*, while the codon showed about 14-fold increased activity in *B. subtilis* than with the UUG start codon. Hence, in both *E. coli* and *B. subtilis*, the most efficient initiation codon for translation of the *xyl*A gene was AUG as expected, in good agreement with previously reported observations [9, 13, 19]. On the other hand, the significant difference between the two organisms in translation initiation efficiency of the initiation codon could be explained by the specificity of translational species. Unexpectedly, UUG was found to be more efficient for translation of the *xylA* gene than GUG in both organisms, contrary to the other systems reported to date [13, 19]. This order of initiation codon preference implies that sequence context around the *xylA* might also influence the efficiency of translation initiation.

**Table 1.** β-Xylosidase activities of initiation codon mutants.

| Initiation codon | Specific activity[a] | |
|---|---|---|
| | *E. coli*[b] | *B. subtilis*[c] |
| UUG | 1.31±0.3 | 6.14±3 |
| AUG | 3.56±1.4 | 84.99±45 |
| GUG | 1.19±0.3 | 4.08±4 |

[a]One unit of enzyme was defined as the amount of enzyme required to release 1 μmole of *p*-nitrophenol per min. The values shown are the averages (±standard deviation) of four independent experiments.

[b]unit/mg.

[c]$10^{-1}$ unit/mg.

### Northern Blot Analysis of the Translation Initiation Codon Mutants

To investigate the cause of the dependence by the *xylA* gene on AUG for its maximum expression in molecular details, Northern blot analysis was first used to determine the amounts of the *xylA* transcripts synthesized in both *E. coli* and *B. subtilis* cells, containing the *xylA* gene with three different initiation codons (AUG, GUG, or UUG).

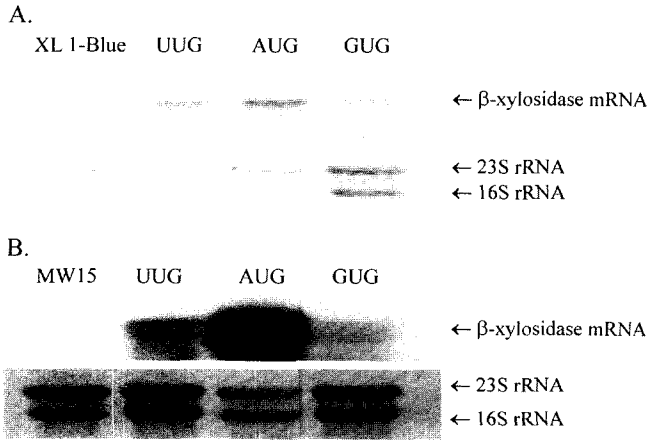


**Fig. 4.** Northern blot analysis in *E. coli* and *B. subtilis*.
(A) Northern blotting in *E. coli*. (B) Northern blotting in *B. subtilis*. The labels in the top position indicate the source of the RNAs. The upper panel indicates the position of full-length *xylA* mRNA, which is assayed as described in Materials and Methods. The lower panel indicates the positions of the 23S and 16S rRNAs.

As shown in Fig. 4, the relative amounts of the *xylA* transcripts synthesized from the three different *xylA* genes showed the pattern similar to those for the β-xylosidase activities. The amount of *xylA* mRNA obtained from the cells containing the *xylA* gene with AUG was higher than those from the cells containing the *xyl*A with UUG or GUG codons both in *E. coli* and *B. subtilis*. However, this does not necessarily imply that the translation initiation codon had any influence on the transcription level. Rather, the results described above could have been related to mRNA stability, as reported in a previous study [9], in which the translation start codon affected the mRNA's ribosome binding strength, and the strength of ribosome binding could influence the frequency of translation. Moreover, the high translation frequency contributed to the mRNA functional stability by possibly protecting it from degradation.

## Efficient *In Vitro* Ribosome Binding of the *xyl*A mRNA with AUG

Next, it was tested whether the greater amount of *xyl*A mRNA which had the AUG start codon was the direct consequence of the ribosome's masking of internal RNase target sites within the mRNA. The stability of *xylA* mRNA was assessed by measuring the mRNA levels in *B. subtilis* cells containing the *xyl*A gene with the three different initiation codons, after the addition of rifampicin (200 μg/ml) to stop further RNA synthesis. As expected, the results showed that the mRNA from the bacterial cells containing the AUG initiation codon was more stable than the mRNAs from the cells containing GUG or UUG codons (Fig. 5A).

In addition, primer extension inhibition (toeprint) assays were carried out to further estimate the relative ribosome binding strength of the *xylA* mRNAs with UUG and AUG start codons (Fig. 5B). mRNAs containing UUG or AUG start codons were translated for 0 or 15 min in *E. coli* S30 extracts (Promega). Oligonucleotide primers for the *xylA* mRNA were added, and the primers were then extended by using reverse transcriptase. The relative intensities of the toeprint signals thus obtained differed significantly with translation initiation codons: The toeprint signal was the strongest in the mRNA containing the AUG initiation codon with polysomes (Fig. 5B, lane 9), whereas a weak signal was observed with the UUG initiation codon (Fig. 5B, lane 6). On the other hand, the toeprint signals were eliminated, when reverse transcription reaction mixtures were incubated for 15 mins with EDTA to disrupt polysomes, and then magnesium was added and primer extension was performed (Fig. 5B, lanes 7 and 10). These toeprinting assays illustrate that the AUG initiation codon was required to produce a toeprint signal with polysome and mRNA.

Therefore, the toeprinting analysis together with the Northern blot analysis demonstrated that the translation start codon contributed to the strength of the ribosome binding site, resulting in a greater abundance of the functional *xylA* mRNA with AUG start codon than the

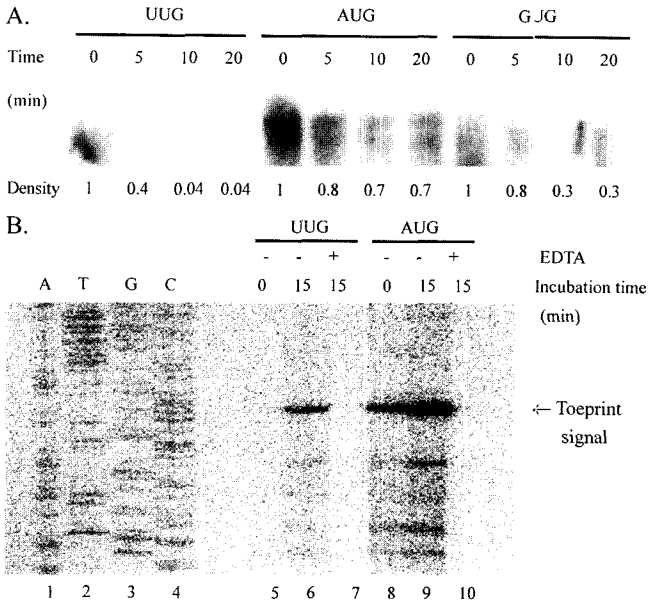


**Fig. 5.** Efficient *in vitro* ribosome binding of RNA from cells containing AUG.
(A) Northern blot analysis of *xylA* mRNA isolated at indicated times (in minutes) after the addition of rifampicin (200 μg/ml). The degree of mRNA stability was indicated in the lower panel, as the quantity of bands with the TINA 2.0 program. (B) Primer extension inhibition (toeprint) assays. Lanes 1 to 4, a sequencing reaction. *xylA* mRNAs containing UUG and AUG were translated for 0 min (lanes 5 and 8) or 15 min (lanes 6, 7, 9, and 10) at 25°C in *E. coli* S30 lysate. EDTA was added to other reactions up to a final concentration of 5 mM (lanes 7 and 10), so as to disrupt polysomes. Magnesium was then added to allow the reverse transcriptase to function, after which primer extension was performed.

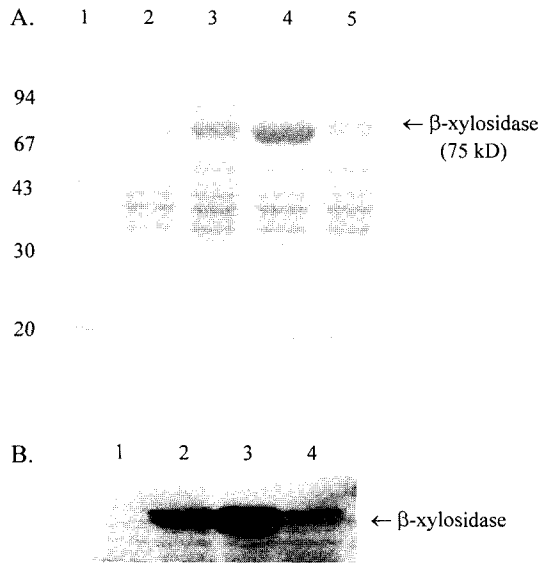


**Fig. 6.** Translational efficiency when *xylA* is mutated from UUG to AUG or GUG.

(A) SDS-PAGE analysis of protein expression in the recombinant *E. coli*. Eight % SDS-PAGE was performed. The recombinant plasmid pET-23b(+) containing the *xylA* gene with UUG initiation codon (lane 3), AUG (lane 4), and GUG (lane 5) was transformed into *E. coli* XL1-Blue cells, and the β-xylosidase polypeptide was obtained from the intracellular fraction. Lane 1, Marker; lane 2, *E. coli* XL1-Blue. The arrow at the side indicates the β-xylosidase, and its molecular weight is 75 kD. (B) Western blot analysis of recombinant *E. coli* cells expressing the *xylA* gene. Eight % SDS-PAGE was performed, and the proteins were probed with monoclonal anti-His antibodies. The arrow indicates the β-xylosidase. The β-xylosidase protein level from the cells containing the *xyl*A with AUG initiation codon (lane 3) was much higher than the levels from the cells containing the *xyl*A mRNAs with UUG (lane 2) or GUG (lane 4). Lane 1, *E. coli* XL1-Blue.

*xylA* mRNA containing GUG or UUG. Also, it has been suggested that a strong ribosome binding site provided ribosome-mediated protection of mRNA from RNase degradation.

### Translational Yields of the Three Different *xylA* Initiation Codons

Finally, synthesis of β-xylosidase in *E. coli* was detected by SDS-PAGE and Western blot analyses (Fig. 6). The recombinant plasmid pET-23b(+) containing the *xylA* gene tagged with 6-His residues at its C-terminus was transformed into *E. coli* XL1-Blue cells, and the β-xylosidase polypeptide in the intracellular fraction was detected using anti-His monoclonal antibody. As evident from the SDS-PAGE, the β-xylosidase protein level from the cells containing the *xyl*A with AUG initiation codon was much higher than the levels from the other *xyl*A mRNAs with two different initiation codons (Fig. 6A). These results were further confirmed by Western blot analysis (Fig. 6B); the *E. coli* cells containing the *xylA* gene with AUG initiation codon showed the highest translational yield among the three different initiation codons.

In summary, the translation start site of the *B. stearothermophilus* No. 236 β-xylosidase gene was identified to be a noncanonical initiation codon UUG. Hence, in this study, a set of amber mutants of the *xylA* gene were constructed and analyzed to specifically test the effects that the three most abundant initiation codons had on the translational yields of the *xylA* gene in both *E. coli* and *B. subtilis* host cells. In both organisms, the highest *in vivo* expression level of the *xylA* gene was observed with mRNA containing an AUG initiation codon, followed by mRNA containing UUG, and then only slight expression with mRNA containing GUG. As evident from the toeprinting and Northern blotting assays, the increased amount of *xylA* gene expression from the mRNA with an AUG initiation codon correlated well with a stronger ribosome binding site and an abundance of *xylA* full-length mRNA. The greater abundance of functional mRNA with the AUG codon compared to the mRNAs with UUG or GUG start codons resulted from the ribosomes, more frequent occupancy of the translation initiation region, thereby more efficiently masking internal RNase target sites. Furthermore, Western blotting showed that the enhanced translational yield by *xylA* mRNA with the AUG initiation codon was directly related to the increased synthesis of XylA polypeptide.

## Acknowledgment

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