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D-Xylose detection in *Escherichia coli* by a xylose binding protein-dependent response

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ABSTRACT

A gene circuit for the controlled expression of a marker gene and for the assay of xylose concentration in *Escherichia coli* has been designed and tested. The *xylF* coding sequence for the xylose binding protein (XBP) was cloned in pT7T318U downstream from the promoter for xylanase A from *B. subtilis* (Pbsu), together with the GFP coding sequence (*gfp*) under the control of the *xylF* promoter, forming the pT7T3-GFP-XBP construct. GFP fluorescence in *Escherichia coli* JW3538-1 *xylF*—transformed with pT7T3-GFP-XBP was approximately 1.4× higher after 520 min growth in the presence of 5 mM xylose than in cells transformed with pT7T3-GFP. Under saturating xylose concentration, flow cytometry analysis showed that all cells resulted in homogeneous populations, and the population with XBP showed a fluorescence greater than that without XBP. Activity of the *xylF* promoter in cells transformed with pT7T3-GFP-XBP was ~40% higher than with the pT7T3-GFP. No response was observed with arabinose and ribose, showing that the expression effects were specific for xylose, demonstrating the potential use of the gene circuit as a biosensor.

1. Introduction

Inducible promoters allow the control of gene expression by external stimuli, and the expression of many of the enzymes and transporters that are associated with metabolic pathways are regulated by sugar specific integrating gene circuits. These gene circuits frequently have a single input–output set that relates the concentration of the inducer to the level of gene transcription (Sohka et al., 2009); therefore, the study and characterization of novel promoters is of fundamental importance not only for the production of heterologous proteins but also in synthetic biology (Slusarczyk et al., 2012).

Xylose is the second most abundant carbohydrate produced in nature and has important applications such as an additive in paint and toothpaste (Martel et al., 2010). Xylose may be converted to xylitol, which is widely used in the food, cosmetic and pharmaceutical industries, or to furfural, which is used as a foundry sand linker, in the refinery of lubricating oil, and as a production intermediate of furan, furfuryl alcohol, and tetrahydrofuran (Gallezot, 2012). Plant cell walls contain large amounts of pentoses, and lignocellulosic biomass in the form of agricultural waste is an attractive source of xylose. In order to achieve a high efficiency in the bioconversion of biomass to high-value products, it is necessary to monitor the production of this sugar (Olofsson et al., 2010), and this requires the development of methods for xylose detection.

The main route for xylose uptake by *Escherichia coli* is via the xylose binding protein (XBP), a periplasmic protein that binds D-xylose with high affinity (Ahlem et al., 1982) and which is a component of the xylFGH carrier system that translocates xylose into the cytoplasm (Cirino et al., 2006). The xylose transporter genes xylFGH are co-regulated by CRP (cAMP Receptor Protein) and by the transcriptional activator XylR, which when bound to D-xylose stimulates expression of the xylFGH operon (Song and Park, 1997). These genes are under the control of the inducible promoter PxylF (Song and Park, 1997). In this study we have investigated the role of XBP in the regulation of the PxylF promoter, and demonstrate an

Abbreviations: XBP, xylose binding protein from *Escherichia coli*; PxylF, promoter xylose inducible from *Escherichia coli* (control gene expression of xylFGH); GFPuv, green fluorescent protein (excitation: 395 nm).

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increased promoter activity in the presence of XBP, and have used this element to create a gene circuit in *E. coli* for specific D-xylose detection.

2. Materials and methods

2.1. Bacterial strains, plasmid construction, and cell culture conditions

Escherichia coli JW3538-1 carrying the XBP gene (xylF) "knock-out" was obtained from the *E. coli* Genetic Resources at Yale CGSC (The Coli Genetic Stock Center, USA). The XBP gene, xylF (GenBankTM accession number 948090), was amplified from *E. coli* DH5α genomic DNA by PCR using primers XBP1 (5′gagcacatATGAAAATAAAGAACATTCTACTCACC 3í) that included the 5′-end of the *xyl*F (uppercase) and restriction site for *Nde*I (underlined), and XBP2 (5′ttaatGGATCCTTACAGCTCGCTCTCTTTGT 3′), including the 3′-end of the *xyl*F (uppercase) and including a *BamH*I site (underlined). The 993-bp PCR product (including the XBP signal peptide) was digested with *Nde*I and *BamH*I, cloned into plasmid pT7BsXA (Ruller et al., 2006) under the control of a constitutive promoter from *Bacillus subtilis*. After full nucleotide sequencing, the xylF was sub-cloned into pT7T3 18U (Amersham Biosciences) using the *Eco*RI and *Bam*HI restriction sites, and the resulting construct was denominated as pT7T3-XBP.

Subsequently, the coding sequence of the green fluorescent protein (GFPuv) was fused to the promoter of the xylFGH operon (Pxylf) by amplification of the PxylF promoter sequence (genomic region: 3728887-3729153) using genomic DNA from *E. coli* K12 as template using primers PXF1 and PXF2. The primer PXF1 (tcgttgcatgcTTGAGATAATTCACAAGTGTGC 3′) included the 5′-end of the PxylF (uppercase) and a restriction site for *Sph*I (underlined), and primer PXF2 (5′ aagttcttctcctttactcatGGTGTAGGGCCTTCTGTAGTT 3′) encoded the 3′-end of the PxylF (uppercase) and included an overlap region of the 5′-end of the GFPuv (underlined). The GFPuv coding sequence was amplified using the vector pGFPuv (Clontech, Mountain View, CA, USA) as template and primers LGFP1 (5′ atgagtaaaggagaagaacttTTCACTGGAG 3′) encoding the 5′-end of the GFPuv (uppercase) and the overlap region with the primer PXF2 (underlined), and LGFP2 (5′ tacgatctagaAGACGAAAGGGCCCGTACG 3′) encoding the 3′-end of the GFPuv (uppercase) and including an *Xba*I site (underlined). Overlap PCR was used to fuse the GFPuv coding sequence and the promoter PxylF, as previously described (Pogulis et al., 2000). The reaction product from the overlap PCR (PxylF + GFP) was digested with restriction enzymes *Sph*I and *Xba*I and ligated to the pT7T3-XBP vector, and the resulting construct was denominated as pT7T3-GFP-XBP (Fig. 1B). For control experiments, a fragment encoding GFPuv alone was ligated into *Sph*I/*Xba*I digested pT7T3 18U and the resulting construct was denominated as pT7T3-GFP.

Culture experiments were performed in tryptone broth (TB) (per liter, 10 g of tryptone and 5 g NaCl) and standard mineral base (SMB) (Stanier et al., 1996) supplemented with thiamine (2×10^{-4}% w/v) at 37 °C unless otherwise noted. Antibiotics (Sigma-Aldritch, São Paulo—SP, Brazil) were used at the following concentrations: ampicillin, 100 μg/ml; kanamycin 40 μg/ml.

2.2. Cell growth and induction studies

To evaluate the effect of xylose on GFPuv expression, *E. coli* strain JW3538-1 harboring the expression vectors were grown in TB liquid medium for 12 h and diluted to an OD_{600} of 0.02 in fresh TB medium containing a defined sugar over a range of concentrations, and 300 μL of this mixture were transferred to separate wells in a 96-well microplate. Cells were grown in a microplate reader (SpectraMax, Molecular Devices, Sunnyvale, CA, USA) at 37 °C over a period of 15 h, during which the fluorescence and OD_{600} were measured every 20 min with constant agitation between readings. For GFPuv fluorescence detection, filters with a 10 nm bandpass at 395 nm and 507 nm were used for excitation and emission detection, respectively. Fluorescence readings were normalized to the OD_{600} values, and all experiments were performed in quintuplicate ($n = 5$). For measurement of intact cell fluorescence emission spectra, cells harboring the expression vectors were grown in TB liquid medium for 12 h and diluted to an OD_{600} 0.02 in 50 mL of fresh TB containing 1% xylose (w/v). After 8 h growth, 1 mL of culture was collected, and after centrifugation the cell pellet was resuspended in phosphate buffered saline (PBS) to an optical density of 0.1. Fluorescence emission spectra were collected using an SLM Aminco model 8100 spectrofluorimeter with sample excitation at 395 nm and emission measured over the range of 450–550 nm. Flow cytometry analysis (FACS–fluorescent assisted cell sorting) was performed on a FACSAria cytometer (Becton, Dickinson and Company, CA, USA) equipped with a 405 nm excitation laser and a 530/30 nm bandpass emission filter. Data colletion and analysis used the FACSDiva (Version 6.1.1.) software, and for each sample, 10000 events were collected at a rate of 500 to 1000 events per second.

2.3. Estimate of promoter strength

Overnight TB cultures of *E. coli* JW3538-1 cells transformed with either pT7T3-GFP or pT7T3-GFP-XBP were used to inoculate 50 mL of SMB medium supplemented with ampicillin, kanamycin and xylose (0.5% w/v), and the cultures were grown at 200 rpm and 37 °C. The OD_{600} was measured using a Cary 50 UV–visible spectrophotometer (Agilent Technologies, Santa Clara, CA) and the fluorescence was monitored in 1.0-cm quartz cuvettes using an SLM Aminco model 8100 spectrofluorimeter. Fluorescence readings performed during the exponential growth phase were plotted as a function of the increase in OD_{600}, and the slope of the resulting line (f_{ss}) represents the change in GFP levels during the exponential-phase. In order to measure the strength of the promoter, a dynamic model was adopted that takes into consideration the synthesis/degradation and oxygen-dependent maturation of GFP. The rate of GFPuv degradation *in vivo* has a half-life greater than 24 h (Alper et al., 2005; Leveau and Lindow, 2001; Strovas and Lidstrom, 2009) and was therefore not considered further; therefore, the rate of GFPuv production (promoter-driven) in the final model is an indirect measure of promoter activity (P) and can be expressed by the following equation:

$$P = fss\left(\mu\left(1 + \frac{\mu}{m}\right)\right) \quad (1)$$

where μ is the cell growth rate, and m is the maturation constant for the oxygen dependent activation of the 4-(p-hydroxybenzylidene)-5-imidazolinone (p-HBI) fluorophore of GFPuv, for which a value of $1.62\ h^{-1}$ was used (Iizuka et al., 2011). P is given as relative fluorescence units per absorbance unit per hour (RFU $OD_{600}^{-1}\ h^{-1}$).

2.4. SMB-agar growth experiments

Ten thousand-fold dilutions of frozen stocks were spread evenly on SMB-agar (1.5% w/v) plates containing ampicilin, kanamycin, and various concentrations of xylose. The plates were incubated at 37 °C for 48 h, and the number of colonies was obtained from the average CFU counts from three plates.

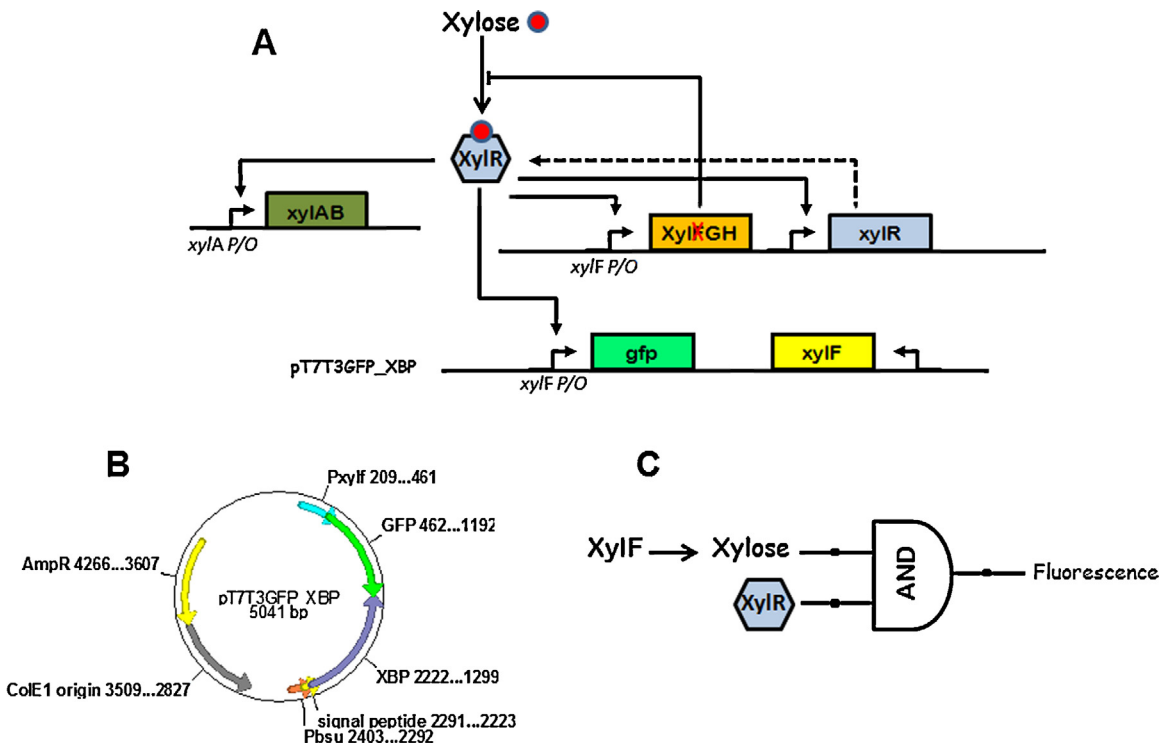

Fig. 1. Regulation of xylose uptake in *E. coli*. (A) The metabolic genes and transporters of xylose are under positive regulation by the XylR/xylose complex. The expression of the transporter XylFGH increases the rate of uptake of xylose, further increasing activation by xylose. In the xylF knockout cell, entry of xylose is impaired. Cells transformed with pT7T3-GFP-XBP containing a copy of active XBP (xylF) take up more xylose, resulting in the increase in GFP expression controlled by XYL P/O (Pxylf) induced by xylose. (B) Schematic depiction of the pT7T3-GFP-XBP plasmid. (C) Conceptualization of the circuit as an AND gate, the increase in fluorescence is obtained in the presence of two input signals: xylose (via XBP) and XylR (activator).

3. Results

3.1. Design and construction of the circuit

A scheme presenting xylose uptake in *E. coli* is shown in Fig. 1A (see the figure legend for details), and on the basis of this knowledge, a simple gene circuit that is sensitive to xylose was constructed and successfully tested in *E. coli* JW3538-1 *xylF*$^{-}$ cells. The XBP knockout cells have a limited xylose uptake, and the vector copy of the *xylF* (under the control of the constitutive promoter Pbsu) complements this deficiency, favouring the formation of the transcriptional activator XylR–xylose complex. The XylR-xylose complex in turn binds to the PxylF promoter, increasing transcription of the GFPuv. The pT7T3-GFP-XBP vector (Fig. 1B) has a ColE1 origin of replication, a high copy number origin of replication yielding 50–70 plasmid copies per cell in log phase (Lutz and Bujard, 1997)) and ampicillin resistance. The circuit can be conceptualized as an AND gate (Fig. 1C) (Hasty et al., 2002), in that the increase in fluorescence is obtained in the presence of two input signals: xylose (in the form of the xylose/XBP complex) and XylR transcription activator.

3.2. Reporter validation and fluorimetry-based gene-expression

E. coli cells transformed with either pT7T3-GFP or pT7T3-GFP-XBP and grown in TB medium with xylose (10 mM) were strongly fluorescent on excitation at 395 nm. This demonstrates that the PxylF promoter can drive GFPuv expression. In all experiments, a higher fluorescence signal was observed in cells transformed with the pT7T3-GFP-XBP as compared to cells transformed with pT7T3-GFP (Fig. 2). The increase in the fluorescence signal in 96-well microplates as a function of time is linear over the period 240–480 min (Fig. 2A). After this period, the fluorescence remained constant (Fig. 2A), showing a 30% increase in fluorescence signal in cells transformed with XBP as compared to control cells. The emission scan over the 480–550 nm emission range with excitation at 395 nm (Fig. 2A inset) shows an emission maximum at 507 nm, which is characteristic of GFPuv (Crameri et al., 1996). The increased fluorescence in the XBP transformed cells was maintained over an OD range of 0.5 to 3.0 (Fig. 2B), showing that the response of the gene circuit was maintained over a range of cell densities, and was propagated over multiple generations.

3.3. Substrate specificity of the PxylF promoter

The inducible GFPuv fusion driven by the PxylF promoter was successfully constructed using overlap extension PCR, and the Pxylf promoter activity was evaluated with increasing concentrations of arabinose, ribose and xylose (Fig. 3A). Over the concentration range tested, ribose and arabinose had no effect on the Pxylf promoter, demonstrating the specificity of the circuit to xylose. The response of the promoter to increasing xylose concentration was linear over the range of 2–10 mM, and is consistent with the results presented in Fig. 2, with an increase of approximately 30% in fluorescence intensity observed in cells transformed with XBP. Although in the presence of 5 mM glucose the effect of xylose induction of GFPuv was significantly reduced, a 70% increase in GFPuv induction was observed in the presence of XBP (Fig. 3B).

A higher growth rate was also observed in cells transformed with XBP in the number of colonies growing on plates containing minimal medium, where the only carbon source was xylose (Fig. 3C). No growth was observed at xylose concentrations of 0–1.3 μM, however at a concentration of 13 μM, a 500% increase in the number of colonies in cells transformed with XBP was observed. On plates with a xylose concentration of 130 μM, the number of colonies transformed with pT7T3-GFP-XBP increased by 25%, and

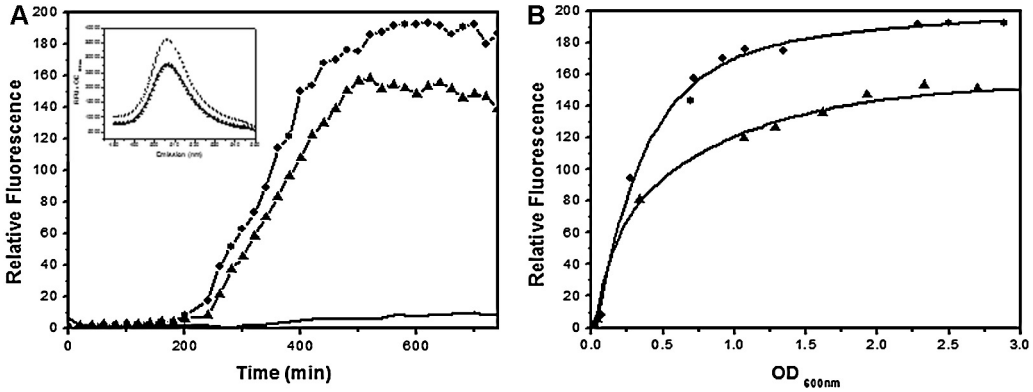

Fig. 2. Fluorescence signal of *E. coli*-K12 ΔxylF cells harboring the expressing vectors pT7T3-GFP and pT7T3-GFP-XBP. (A) Time course of the fluorescence increase of cells transformed with pT7T3-XBP (control without GFP, black line), pT7T3-GFP-XBP (circles) or pT7T3-XBP (triangles). (B) Relationship between cell fluorescence and growth. Results shown in A and B are values obtained of growth in TB medium in 96-well microplates (see Section 2 for further details). The inset to panel A shows the fluorescence emission spectra from cells transformed with pT7T3-GFP-XBP (circles) or pT7T3-XBP (triangles).

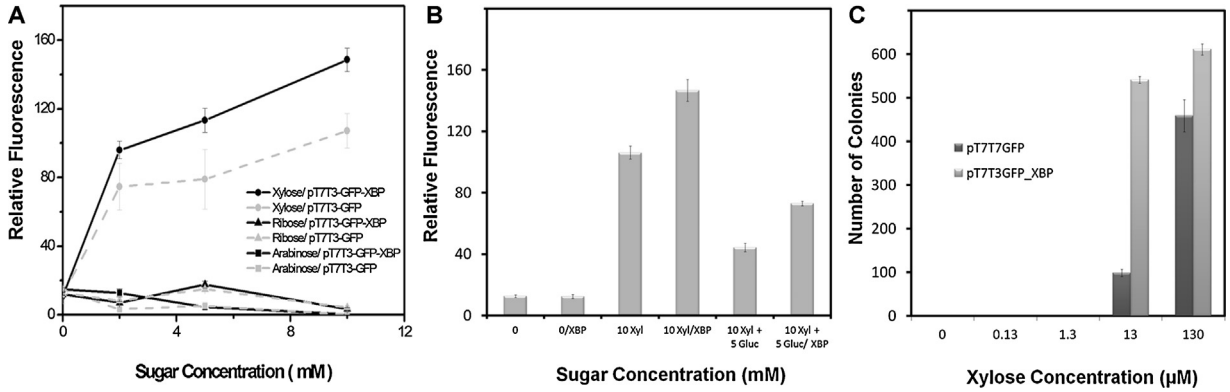

Fig. 3. Comparison of PxylF promoter activity in response to different sugars in *E. coli*-K12 ΔxylF cells harbouring the vectors pT7T3-GFP and pT7T3-GFP-XBP. (A) Effect of increasing the concentration of the pentoses arabinose, ribose and xylose. (B) Effect of glucose (Gluc) addition. Cells harbouring either pT7T3-GFP-XBP (XBP) or pT7T3-GFP in the presence of xylose (Xyl) and glucose (Gluc). (C) Effect of concentration of xylose on solid minimal medium in low xylose concentration. Results are values obtained after 400 min of growth in 96-well microplates and the averages and standard deviation of five repetitions ($n = 5$).

these colonies had larger diameters. At higher xylose concentrations, it is likely that alternative mechanisms of xylose uptake by low-affinity transporters can maintain the carbon supply to the cells, and transport via XBP is no longer essential, decreasing the difference between the cells transformed with and without the XBP.

3.4. Flow cytometry analysis

FACS detection and analysis was used to investigate the distribution of cells in bulk cultures in terms of relative size (estimated by forward scatter analysis) and fluorescence intensity (shown in Supplementary Fig. S1). The cultures containing the pT7T3-XBP, pT7T3-GFP and pT7T3-GFP-XBP plasmids all presented homogeneous growth (Fig. S1). All cultures were induced homogeneously, and the mean fluorescence intensity of the cells expressing the XBP was about 2.5-fold higher than that of the control cells (mean fluorescence intensities for pT7T3-GFP-XBP = 6511 and pT7T3-GFP = 2522 au) (Fig. 4).

3.5. Estimation of promoter activity

The $\Delta RFU/\Delta OD_{600}$ ratio was determined for cultures growing in SMB medium. A linear relationship between relative fluorescence units (RFU) and cell density (OD_{600}) was observed for two transcriptional fusion strains (Fig. 5A). The promoter activities for strains with and without XBP were calculated as described in Section 2 (Table 1), and showed a ~40% higher higher PxylF promoter activity in cells transformed with pT7T3-GFP-XBP as compared to cells transformed with pT7T3-GFP. This difference in activity reflects both the difference in the steady state GFPuv fluorescence (*fss*), which is ~20% higher for cells expressing XBP (Fig. 5A and Table 1), and the growth rate (μ), which was ~15% higher for cells with XBP

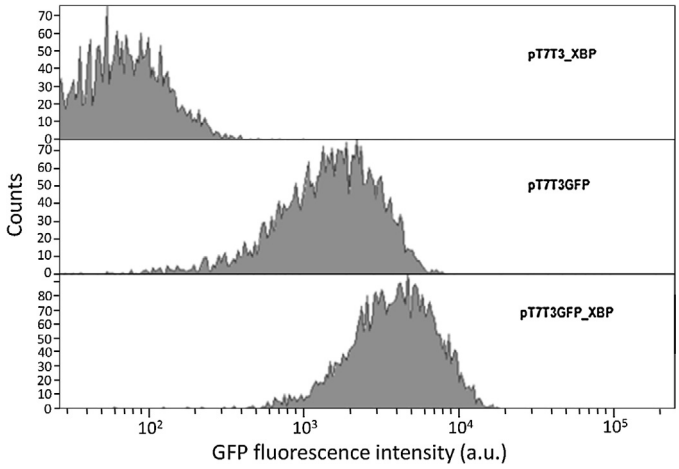

Fig. 4. FACS histograms of GFPuv expression in populations of *E. coli* cells transformed with pT7T3-XBP (upper panel), pT7T3_GFP (middle panel) and pT7T3_GFP_XBP (lower panel). The three different cell samples were measured after 400 min of induction. See Section 2 for further details.

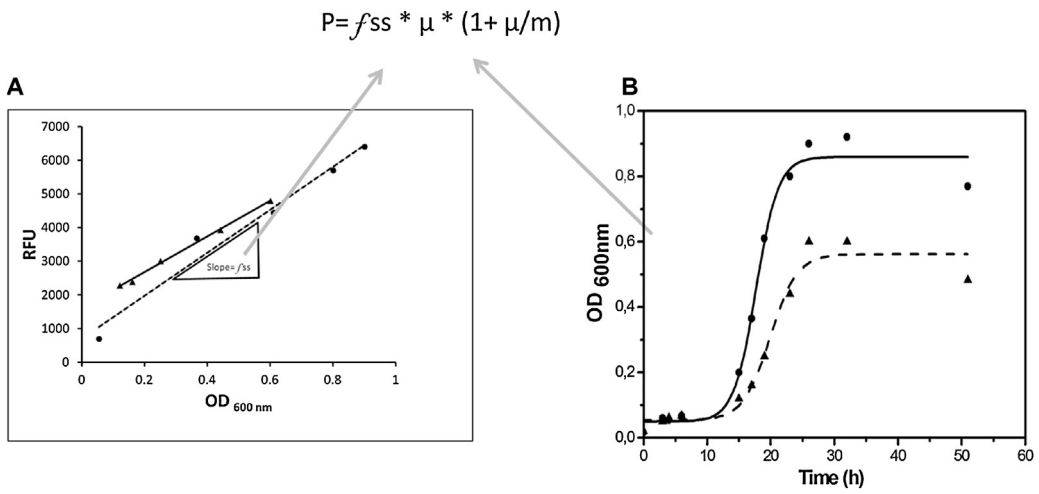

Figure 5. Estimation of PxylF promoter activity. (A) Determination of the steady state fluorescence (as relative fluorescence units, RFU) in increasing concentrations of xylose. (B) Growth curve, *P* = promoter activity (RFU $OD600^{-1}$ h^{-1}); *fss* steady-state fluorescence ($\Delta RFU/\Delta OD_{600}$); μ = growth rate (h^{-1}); *m* = maturation half-life of GFPuv (0.45 h^{-1}). Results are the values obtained of growth in 50 mL of minimal medium (SMB) containing 1% xylose. The symbols in both panels of this figure are; ● pT7T3-GFP-XBP; ▲ pT7T3-XBP.

(Fig. 5B and Table 1). These results indicate that XBP enhanced entry of xylose into the cell, resulting in an increase of approximately 40% in the transcription of the gene regulated by Pxylf.

4. Discussion

Accumulated knowledge with respect to gene regulation has not only led to increasingly sophisticated control of existing cellular processes, but also allows the introduction of novel functions and has led to the emergence of the discipline of synthetic biology. Gene circuits are the fundamental modules which can perform relatively simple functions from which more complex integrated networks may be constructed that are capable of performing increasingly elaborate functions such as switches, oscillators, filters and cell–cell communication (Khalil and Collins, 2010; Kitney and Freemont, 2012; Nandagopal and Elowitz, 2011; Stein, 2012; Voigt, 2012). Reprogramming cells to act as sensors for biomolecules is a potentially useful application of synthetic biology, and gene circuits for the detection of mechanical, electrical, and chemical stimuli have previously been constructed (Lu et al., 2009).

Understanding the regulation of known promoters together with the discovery of new inducer–promotor systems is of fundamental importance for the advancement of synthetic biology. The xylA and xylF promoters are key elements in the regulation of xylose operons in *E. coli*, and both are regulated by xylose (Desai and Rao, 2010). The XylR protein controls expression of the xylose operon in *E. coli*, and acts positively in the presence of xylose and negatively in its absence (Groff et al., 2012; Song and Park, 1997). The xylA promoter is well characterized and has been used in various applications (Bhavsar et al., 2001; Desai and Rao, 2010; Groff et al., 2012); however, there are few reports of xylF promoter regulation. One of the aims of the present work is the characterization of the PxylF promoter with respect to the periplasmic xylose binding protein (XBP). In the present study, we have shown that the expression of GFP in cells transformed with pT7T3-GFP-XBP (which contains the xylF gene encoding XBP under constitutive Pbsu promoter control) was consistently higher than in cells transformed with pT7T3-GFP (which does not contain xylF) under different conditions using different measurement methods. This difference in the fluorescence signal between cells containing the XBP gene (pT7T3-GFP-XBP) and cells lacking XBP (pT7T3-GFP) was due to GFP expression and not to cell growth, since cells carrying XBP showed higher fluorescence at the same OD_{600} (Fig. 2B) and in single cell analysis (Fig. 4). Flow cytometry analysis showed that cells deficient for the transport of xylose, both with and without the xylF gene at saturating xylose concentration (10 mM), resulted in homogeneous populations (Fig. 4); however, cells transformed with XBP showed increased fluorescence. Growth in microplates of the cells with XBP showed 1.4-fold greater fluorescence than cells without XBP, and in flow cytometry experiments, this difference increased to 2.5-fold (mean fluorescence intensity for pT7T3-GFP-XBP = 6511 and pT7T3-GFP = 2522). Culture conditions with high aeration, such as used in the flow cytometry experiments, may result in an increase in the GFPuv levels in the XBP culture when compared to growth in microplates under conditions of low agitation. Oxygen is required for the final oxidation step of the phenyl side chain of Tyr66 to form the mature chromophore in the GFP protein (Iizuka et al., 2011), and the efficiency of this step depends on time, temperature, oxygen availability, and rates intrinsic cyclization/oxidation during chromophore formation (Cubitt et al., 1995; Tsien, 1998).

The xylF promoter (PxylF) demonstrates several characteristics that favor its use in the construction of gene circuits, such as specificity for xylose and a concentration-dependent response (Fig. 3A). In the presence of glucose, the GFPuv expression decreased (Fig. 3B) due to the diauxic growth characteristics of *E. coli*, where glucose is preferentially assimilated before xylose as a result of cyclic AMP receptor protein (CRP)-dependent control of xyl genes (Cirino et al., 2006; Desai and Rao, 2010; Song and Park, 1997). However, even though the fluorescence intensity decreases in the presence of glucose, the difference between the fluorescence signals in the cultures

Table 1
Activity parameters of the PxylF promotor fused with GFPuv in *E. coli*-K12 Δ xylF.

Construction	Promoter	μ (h^{-1})	t_g (min)	*fss* $\Delta RFU/\Delta OD_{600}$	*m* (h^{-1})	*P* (RFU OD_{600}^{-1} hr^{-1})
pT7T3-GFP	Pxylf	0.223	185.6	5290.5	1.62	1342
pT7T3-GFP-XBP	Pxylf	0.257	161.1	6284.1	1.62	1871

increased from ~30% in the absence of glucose (108.1 to 142.6 RFU) to ~70% in the presence of glucose (43.4 to 74.1—Fig. 3B), which suggests that xylose entry continues as the result of the constitutive expression of XBP. In the analysis of cell growth in which xylose was the sole carbon source, the system was more sensitive to changes in the concentration of xylose (Fig. 3C). However, at higher concentrations, xylose enters the cell by less specific mechanisms and difference in the responses between the constructs decreases. The greater sensitivity to low concentrations of xylose, which is reflected in the number of cells, suggests a potential application of the circuit for the detection of xylose at low concentrations.

The rapid maturation of GFPuv is a desirable property for monitoring the activity of promoters. Using a dynamic equation balancing GFPuv production and degradation (formula (1)), promoter activity P can be estimated from experimentally determined values for RFU/OD and μ. The difference of approximately 40% between the activity of the PxylF promoter in cells with and without XBP resulted from a balance between cell growth (μ) and fluorescence per cell unit (RFU/OD). This work has shown that the latter made the greater contribution, and we conclude that the effect of XBP was to increase the XylR/xylose complex concentration, which in turn activates gene expression. Previous studies have frequently shown that an increase in GFP marker fluorescence is greater than the increased activity resulting from promoter induction (Alper et al., 2005; Leveau and Lindow, 2001), and is a consequence of the fact that a single mRNA can be translated several times to produce multiple polypeptides. This is in agreement with the results in the current work, in which the flow cytometry shows a 2.5-fold fluorescence increase, whereas the promotor activation increases by only 30%.

In summary, microbial circuits for detecting xylose have potential in control processing of natural materials rich in xylose, such as lignocellulose. Such applications involve selective detection of xylose in mixtures with other sugars. Taking into account the characteristics of the promotor described in the current work, the gene circuit that has been designed, constructed and characterized may have applications as a xylose biosensor. Furthermore, continued application and study of this promoter may facilitate efforts in synthetic biology with the goal of creating synthetic genetic operons. The availability of the PxylF promotor element, together with the characterization reported here may help in the selection of components for use in synthetic genetic circuits with defined ratios of gene expression.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jbiotec.2013.10.019.

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