Data Article

Data on the standardization of a cyclohexanone-responsive expression system for Gram-negative bacteria

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A B S T R A C T

Engineering of robust microbial cell factories requires the use of dedicated genetic tools somewhat different from those traditionally used for laboratory-adapted microorganisms. We have edited and formatted the ChnR/PchnB regulatory node of Acinetobacter johnsonii to ease the targeted engineering of ectopic gene expression in Gram-negative bacteria. The proposed compositional standard was thoroughly verified with a monomeric and superfolder green fluorescent protein (msfGFP) in Escherichia coli. The expression data presented reflect a tightly controlled transcription initiation signal in response to cyclohexanone. Data in this article are related to the research paper “Genetic programming of catalytic Pseudomonas putida biofilms for boosting biodegradation of haloalkanes” [1].

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**VALUE OF THE DATA**

- Standardized vector designed for tightly regulated gene expression in Gram-negative bacteria.
- Regulatory elements from *Acinetobacter johnsonii* (ChnR transcriptional regulator and P:\textsubscript{chnB} promoter) edited, formatted, and assembled in a minimal DNA segment adopting a Synthetic Biology standard.
- Responsiveness of the expression system to the inducer cyclohexanone demonstrated by using GFP as a reporter.
- The DNA standard described in this dataset could be used as a benchmark for future research on gene expression in Gram-negative bacteria.

1. **Data**

A cyclohexanone-responsive expression platform was designed based on elements of the cyclohexanol biodegradation pathway of *Acinetobacter johnsonii* (Fig. 1A). The segments bearing the complete chnR and P:\textsubscript{chnB} promoter DNA of *A. johnsonii* NCIMB 9871 were edited in silico to obtain a standardized SEVA (Standard European Vector Architecture [2]) expression cargo (Fig. 1B), and assembled to yield plasmid pSEVA2311 (Fig. 2 and Table 1). Expression data were generated to validate this plasmid. The gene encoding the monomeric and superfolder green fluorescent protein (msf\textsubscript{GFP}) was inserted in vector pSEVA2311 (Fig. 3A), and the transcriptional activation of the ChnR/ P:\textsubscript{chnB} expression system upon addition of cyclohexanone was evaluated in a wild-type *Escherichia coli* strain (Fig. 3B and C). Key features of this expression vector include [i] a very low expression level in the absence of inducer, [ii] high transcriptional capacity, [iii] an induction kinetics very similar in both minimal and rich culture media, and [iv] linear accumulation of the reporter product along time.

2. **Experimental design, materials and methods**

2.1. **Bacterial strains and culture conditions**

Bacterial strains used in this study are listed in Table 1. *E. coli* CC118 was used as the host for plasmid constructs and it was routinely grown at 37 °C in LB medium [3]. For single-cell fluorescence determination by flow cytometry, cells were grown in the semi-synthetic M9CAG medium, which contains the same salts as for M9 minimal medium [3], 0.1% (w/v) acid casein hydrolysate (Becton-Dickinson Diagnostics Co., Sparks, MD, USA), 2 mM MgSO\(_4\), 0.1 mM CaCl\(_2\), 0.05% (w/v) vitamin B1, and 0.4% (w/v) glucose as the sole carbon and energy source. Kanamycin (Km, 50 μg ml\(^{-1}\)) was added to the culture media whenever required. Growth was estimated by measuring the optical density at 600 nm (OD\(_{600}\)) after diluting the culture whenever needed [4–6]. Shaken-flask cultures were set in 125-ml Erlenmeyer flasks containing culture medium up to one-fifth of their nominal volume and
continuously agitated at 170 rpm. Cyclohexanone was directly added to the cultures as an inducer of the ChnR/PchnB system at 1 mM.

2.2. DNA techniques, plasmid design and construction, and validation of a standardized expression system with regulatory parts from Acinetobacter johnsonii NCIMB 9871

All the plasmids used in this study are listed in Table 1. DNA amplification by PCR, digestion with restriction enzymes, ligation, and other standard cloning procedures followed well established protocols [3,7–9] and specific instructions from the manufacturers. All plasmid constructs were confirmed by Sanger DNA sequencing (Secugen SL, Madrid, Spain).
To obtain vector pSEVA2311, an expression plasmid containing the $P_{\text{chnB}}$ promoter and the gene encoding the cyclohexanone-responsive ChnR transcriptional regulator, a DNA fragment carrying both $\text{chnR}$ and $P_{\text{chnB}}$ was designed as a SEVA expression cargo (i.e., $\text{PacI}/\text{AvrII}$), and synthesized de novo by GeneCust Europe S.A. (Dudelange, Luxembourg). The DNA sequence encoding ChnR, including a putative binding site for ChnR upstream to the $P_{\text{chnB}}$ promoter, was taken from the genome of $A.\ johnsonii$ NCIMB 9871 [10–12]. The recognition site for $\text{HindIII}$, $\text{PacI}$, and $\text{PstI}$ in this synthetic DNA fragment were manually edited to erase them, as these restriction targets are present in the multiple cloning site of all pSEVA plasmids (Fig. 1B). A DNA fragment was amplified using oligonucleotides $\text{chnR}-F$ (5'-TTT TTT TTT AAT TAA TCA AAA AAC AAT AGA GGA GAC TGA ATT TTC-3', recognition site for $\text{PacI}$ underlined) and $\text{chnR}-R$ (5'-TTT TGC TAG CAT GAG CAC AGA CAA AGC AAA TAC-3', recognition site for $\text{Nhel}$ underlined) from the synthetic DNA fragment described above, and sub-cloned into vector pSEVA231 as a $\text{PacI}/\text{Nhel}$ fragment. The constitutive expression of $\text{chnR}$ was achieved by inserting a $\text{Nhel}/\text{AvrII}$ fragment that spans a 150-bp long linker sequence and the strong, constitutive $P_{\text{kan}}$ promoter [13] along with the $\text{tir}$ motif (5'-GAT TAA TCT TAT AAG GAG GAA AAA-3' [14]), giving rise to the intermediate plasmid pSEVA231-ChnR (Table 1). This vector was inserted with the $P_{\text{chnB}}$ Promoter, originating pSEVA2311 (Fig. 2 and Table 1). Another version of the expression vector was also constructed, but using a plasmid backbone bearing the pRO1600/ColE1 origin of replication, giving rise to pSEVA2411 (Table 1). For the experimental validation of the ChnR/$P_{\text{chnB}}$ expression system, the gene encoding the monomeric and superfolder green fluorescent protein (msf-GFP) was excised from plasmid pSEVA237M as a $\text{HindIII}/\text{SpeI}$ fragment and cloned into pSEVA2311, giving rise to plasmid pSCM (Table 1 and Fig. 3).

### 2.3. Single-cell analysis by flow cytometry

A MACSQuant™ VYB cytometer (Miltenyi Biotec, Bergisch Gladbach, Germany) was used for msf-GFP analysis and quantification as indicated elsewhere [15]. An Ar laser, diode-pumped solid state, was used to excite msf-GFP at 488 nm and the fluorescence signal was recovered with a 525 ± 40 nm band-pass filter. Plasmid pSCM was introduced in wild-type $E.\ coli$ BW25113 by chemical transformation [3]. Recombinant cells were grown overnight in semi-synthetic M9CAG medium with the appropriate carbon source and antibiotics, diluted 1/100 in fresh M9CAG culture medium (initial OD$_{600}$ of ca. 0.05), and further incubated until the cultures reached the mid-exponential phase (OD$_{600}$=0.5). At this point, cells were divided into two samples; one of them was...

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**Table 1**

Bacterial strains and plasmids used in this study.

| Bacterial strain | Relevant characteristics* | Reference |
|------------------|---------------------------|-----------|
| **Escherichia coli** |                         |           |
| CC118            | Cloning host; $\Delta(\text{ara-leu})$ $\text{araD}$ $\Delta\text{lacX174}$ $\text{galE}$ $\text{galK}$ $\text{phaA}$ $\text{thiE1}$ $\text{rpsE}$ $\text{rpoB}^{\text{Rif}^*}$ $\text{argE(Am)}$ $\text{recA1}$ | [19] |
| BW25113          | Wild-type strain; F $^+\Delta(\text{araD-araB})\cdot567$ $\Delta\text{lacZ4787}\cdot(\text{rrnB-3})$ $\text{rph-1}$ $\Delta(\text{rhaD-rhaB})\cdot568$ $\text{hsdR}514$ | [20] |
| **Plasmids**     |                         |           |
| pSEVA231         | Km$^\circ$; orfV(pBBR1), standard multiple cloning site | This study |
| pSEVA231- ChnR   | Km$^\circ$; pSEVA231 carrying $\text{chnR}$ (encodes the ChnR transcriptional regulator from *Acinetobacter johnsonii*) | This study |
| pSEVA237M        | Km$^\circ$; orfV(pBBR1), promoterless msf-GFP | This study |
| pSEVA2311        | Km$^\circ$; orfV(pBBR1), $\text{chnR}$, $P_{\text{chnB}}$: standardized, cyclohexanone-responsive expression vector | This study |
| pSEVA2411        | Km$^\circ$; orfV(pRO1600/ColE1), $\text{chnR}$, $P_{\text{chnB}}$: standardized, cyclohexanone-responsive expression vector | This study |
| pSCM             | Km$^\circ$; orfV(pBBR1), $\text{chnR}$, $P_{\text{chnB}}$→msf-GFP | This study |

* The abbreviations used in this table are as follows: Km, kanamycin; Sm, streptomycin; Rif, rifampicin; msf-GFP, gene encoding the monomeric and superfolder green fluorescent protein (msf-GFP).
induced by addition of cyclohexanone at 1 mM, and the other was kept as a non-induced control. Cultures were further incubated as described above and an aliquot of each sample was withdrawn each hour after the induction point, and stored on ice until analysis. The flow cytometry analysis was executed on at least 20,000 cells and the data was processed using FlowJo v. 9.6.2 software (FlowJo LLC, Ashland, OR, USA) [16]. Data regarding expression levels and induction kinetics for *E. coli* strains carrying plasmid pSCM were similar in LB medium and M9CAG medium (data not shown).
2.4. Chemicals and reagents

Unless stated otherwise, all chemicals and inducers were purchased from Sigma-Aldrich Co. (St. Louis, MO), while flow cytometry materials (buffers and calibration beads) were purchased from Miltenyi Biotec GmbH.

3. Material availability and repository

The expression vectors described in this work are part of the SEVA initiative (http://wwwuser.cnb.csic.es/~seva/, [2,17]) and are available free of charge upon request.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2016.01.022.

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