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Engineered fumarate sensing *Escherichia coli* based on novel chimeric two-component system

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ABSTRACT

DcuS/DcuR two component system (TCS) was firstly employed for the expression of the *gfp* gene under the *dcuB* gene promoter in aerobic condition to develop high throughput screening system able to screen microorganisms producing high amount of fumarate. However, the DcuS/DcuR TCS could not produce a signal strong enough to mediate the expression of the *gfp* gene responding fumarate concentration. Thus, DcuS/DucR TCS was engineered by recruiting the EnvZ/OmpR system, the most-studied TCS in *E. coli*. A chimeric DcuS/EnvZ (DcuSZ) TCS was constructed by fusing the sensor histidine kinase of DcuS with the cytoplasmic catalytic domain of EnvZ, in which the expression of the *gfp* gene or the *ompC* gene was mediated by the *ompC* gene promoter through the cognate response regulator, OmpR. The output signals produced by the chimeric DcuSZ TCS were enough to detect fumarate concentration quantatively, in which the expressions of the *gfp* gene and the *ompC* gene were proportional to the fumarate concentration in the medium. Moreover, principal component analysis of C_4-dicarboxylates showed that DcuSZ chimera was highly specific to fumarate but could also respond to other C_4-dicarboxylates, which strongly suggests that TCS-based high throughput screening system able to screen microorganisms producing target chemicals can be developed.

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1. Introduction

C_4-dicarboxylates (aspartate, fumarate, malate and succinate) are core metabolites that serve as alternative substrates to hexose as a carbon source for *Escherichia coli* (Unden and Kleefeld, 2004). Fumarate is one such C_4-dicarboxylate and is an intermediate in the TCA cycle. It has many uses, including therapeutic administration to psoriatic individuals in the form of fumaric acid monoethyl or dimethyl ester. In addition, a large reduction (up to 70%) in methane emissions from cattle can be achieved by providing a fumaric acid-based additive as a supplement in their diet. Fumaric acid, together with the related succinic acid and malic acid, has been identified as one of the top ten building block chemicals that can be produced from sugars via chemical or biological conversion (Lee et al., 2004). Fumaric acid could be more suitable than other carboxylic acids in the polymer industry because it is non-toxic. It can be used as a starting material for polymerization and esterification reactions (Domb et al., 1990; Jaćović et al., 1992). In addition, greater hardness of the polymer structure can be achieved by using fumarate (Roa Engel et al., 2008).

A novel fumarate sensing system is needed to enable high-throughput screening of fumarate overproducing microorganisms. Bacterial biosensors are considered one promising candidate for this purpose. Bacteria sense environmental stimuli or stress using a two-component system (TCS), which consists of histidine kinase (HK) and a response regulator (RR) (Stock et al., 2000; West and Stock, 2001). HK is a transmembrane protein with the N-terminal sensor domain in the extracytoplasmic compartment (periplasm, inner or outer membrane, or even extracellular space) and the C-terminal autokinase domain in the cytosol (Dutta et al., 1999; Parkinson, 1993; Stock et al., 2000). The RR consists of a regulatory domain that includes a phospho-accepting aspartate residue at the N-terminal region and an associated effector domain containing a DNA-binding motif, which is activated by phosphorylation.

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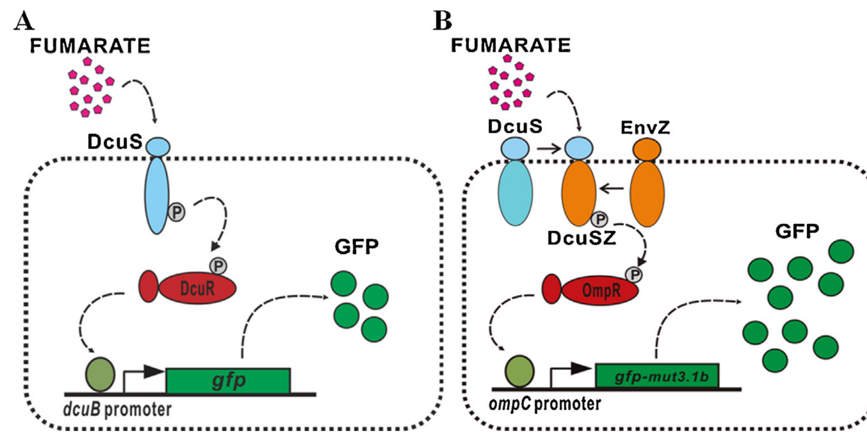

Fig. 1. *E. coli* engineered to respond to exogenous fumarate. (A) A periplasmic receptor (DcuS) senses fumarate, which phosphorylates its histidine kinase domain, and the response regulator (DcuR) of TCS leads to activation of the *dcuB* promoter resulting in GFP expression in *E. coli*. (B) The *E. coli* periplasmic histidine kinase domain of DcuS and the cytoplasmic catalytic domain of EnvZ were fused to form the DcuSZ chimeric protein, which phosphorylates the response regulator OmpR of the TCS while sensing fumarate. This activates the *ompC* promoter, resulting in GFP expression in *E. coli*.

DcuS/R is a C_4-dicarboxylate sensing TCS apparatus. DcuS is the integral membrane sensor HK that belongs to the CitA family of protein kinases and it activates DcuR. The *dcuB* gene is strongly expressed under anaerobic conditions via activated DcuR while the *dctA* gene is weakly expressed under aerobic conditions (Davies et al., 1999; Golby et al., 1999; Zientz et al., 1998). In addition to its weak signal, *dctA* is not an appropriate aerobic fumarate sensor, as it also responds to L-malate, L-aspartate L-tartrate and orotate. Therefore a bacterial system based on the *dcuB* gene is more desirable in monitering the external fumarate concentration compared with that based on the *dctA* gene in the aspects of sensitivity and selectivity toward fumarate.

The EnvZ/OmpR system is the most-studied TCS in *E. coli* that regulates the expression of outer membrane porins, OmpF and OmpC, in response to osmolarity changes (Forst and Roberts, 1994; Egger et al., 1997). The exact mechanism underlying how the expression of outer membrane porin genes *ompC* and *ompF* is triggered by EnvZ/OmpR TCS has not yet been identified (Aiba and Mizuno, 1990; Aiba et al., 1989; Forst et al., 1987, 1989; Igo and Silhavy, 1988). In spite of its unknown mechanism, it has been reported that the function of the EnvZ/OmpR TCS can be engineered by fusion of EnvZ with another HK.

Periplasmic sensing HKs represents the largest group of membrane bound sensor kinases. The HKs are grouped primarily based on the periplasmic sensor domain and linker domain but not by transmitter domain. A chimeric HK can be constructed by integrating two domains from different HKs. A typical example of a TCS chimera is the chimeric receptor Taz (Utsumi et al., 1989). This receptor consists of part of the aspartate receptor of *E. coli* (Tar) fused to the C-terminal part of the *E. coli* osmosensor, EnvZ, which activates transcription of the *ompC* gene in response to aspartate (Weerasuriya et al., 1998).

In this study, the expression of the *gfp* gene under the *dcuB* promoter induced aerobically by *E. coli* DcuS/R TCS was monitored (Fig. 1A). A DcuSZ chimeric HK was also constructed by fusing the periplasmic sensor domain of DcuS with the cytoplasmic catalytic domain of EnvZ (Fig. 1B). The resulting DcuSZ chimeric HK aerobically senses extracellular fumarate and activates OmpR, which induces the expression of the *ompC* gene and the *gfp* gene under the *ompC* gene promoter. The expression profile of the *ompC* gene was monitored by real-time quantitative PCR (qRT-PCR) and green fluorescent protein (GFP) under aerobic condition in order to assess the dynamic response of the chimeric TCS in the presence of fumarate and other C_4-dicarboxylates.

2. Materials and methods

2.1. Bacterial strains and culture conditions

E. coli XL1-Blue and BL21(DE3) strains were used as host strains for recombinant DNA manipulation and the expression of the recombinant proteins. The bacterial strains and plasmids used in this study are listed in Table 1. *E. coli* strains were grown aerobically in Luria–Bertani (LB) broth (10 g/L bacto-tryptone, 5 g/L bacto-yeast extract and 5 g/L NaCl) and in M9 minimal salts medium (Sigma) supplemented with 4 g/L of glucose, 2 mM $MgSO_4$, 0.1 mM $CaCl_2$ and 1% thiamine HCl at 37 °C under vigorous shaking.

2.2. Construction of the plasmid for the expression of the gfp gene under the dcuB gene promoter

Primers for the amplification of the *dcuS*, *envZ*, *dcuB* promoter, and *ompC* promoter genes were designed based on the reported *E. coli* genome sequence (Blattner et al., 1997) and primers for the

Table 1
List of bacterial strains and plasmids used in this study.

Strain/plasmid	Relevant genotype and/or property	Source
***Escherichia coli* strains**		
BL21 (DE3)	*F^- ompT gal dcm lon hsdS$_B$($r_B{}^-$ $m_B{}^-$) λ(DE3 [lacI lacUV5-T7 gene 1 ind1 sam7 nin5])*	Novagen
XL1-Blue	*SupE44 hsdR17 recA1 endA1 gyrA96 thi relA1 lac F′(proAB$^+$ lacI^q lacZΔM15 Tn10 (tet^R))*	Stratagene
Plasmids		
pUC19	Amp^R	New England Biolabs
pDBGFP1	pUC19 containing the *dcuB* promoter and *gfp* gene, Amp^R	This work
pOGFP1	pUC19 containing the *ompC* promoter and *gfp* gene, Amp^R	This work
pACYCDuet-1	Cm^R	Novagen
pDcuSZ1	pACYCDuet-1 containing the chimeric *dcuS-envZ* gene, Cm^R	This work

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