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Development of a whole-cell biosensor for detection of antibiotics targeting bacterial cell envelope in *Bacillus subtilis*

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Abstract

It is an urgent need to develop novel antibiotics to treat infections caused by multi-drug-resistant bacteria. One promising strategy could be the use of whole-cell biosensors, which have been extensively studied to monitor environmental pollutants and intracellular metabolites. Here, we used the σ^M-mediated regulatory system of *Bacillus subtilis* to construct a whole-cell biosensor for the detection of cell envelope-acting antibiotics. Using polymyxin B as the inducer for bacterial cell envelope stress and enhanced green fluorescent protein (EGFP) as the reporter, we found that the promoter of *ypuA* (P_{ypuA}) had the lowest background noise and the most significant changes in the fluorescence output. The whole-cell biosensor displayed dose-dependent and time-dependent responses in fluorescence signals. The detection range of this biosensor for polymyxin B was between 0.125 and 12 μg/mL. The response of the biosensor is specific to antibiotics that target the cell envelope. Besides determination in liquid cultures, the output signal of the biosensor can be easily determined on agar surfaces. Using this biosensor, we successfully detected polymyxins secreted by its producing strain and bacteria that produce cell envelope-acting antibiotics.

Key points

- *A whole-cell biosensor was constructed based on the σ^M-mediated regulatory system.*
- *The response of the biosensor is specific to cell envelope-acting antibiotics.*
- *The biosensor can be used to screen novel cell envelope-acting antibiotics.*

Keywords Whole-cell biosensor · Cell envelope stress response · Extracytoplasmic function (ECF) σ factors · Cell envelope-acting antibiotics · *Bacillus subtilis*

Introduction

The irrational use and even abuse of antibiotics lead to the emergence and spread of antibiotic resistance, which seriously threatens global public health (Blair et al. 2015; Laxminarayan et al. 2013). In the past decades, intensive efforts have been made to discover novel antibiotics from natural products (Hutchings et al. 2019; Pejin et al. 2014; 2017). Recent advances in synthetic biology facilitate the development of new approaches for antibiotic discovery (Atanasov et al. 2021). Among them, whole-cell biosensors show tremendous potential for the screening of novel antibiotics (Lautenschläger et al. 2020; Rebets et al. 2018; Wolf and Mascher 2016). Whole-cell biosensors are genetically engineered microorganisms that link a specific input to a quantifiable output (Yagi 2007). An increasing number of whole-cell biosensors have been designed to monitor environmental pollutants (Belkin 2003; Shin 2011; Jia et al. 2019) and intracellular metabolites (Rogers and Church 2016; Baumann et al. 2018).

The bacterial cell envelope is a multilayered structure for bacteria to adapt to various growth conditions in the changing environment. Gram-positive bacteria and Gram-negative bacteria have different cell envelopes (Jordan et al. 2008; Silhavy et al. 2010). The former consists of cytoplasm membrane, peptidoglycan, and teichoic acid, while the latter is composed of cytoplasm membrane, peptidoglycan, and outer membrane. The bacterial cell envelope can serve as an important target for many antibiotics, such as β-lactams (e.g., ampicillin), glycopeptides (e.g., vancomycin), polymyxins, bacitracin, and fosfomycin (Kohanski et al. 2010;

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Yin et al. 2020). Their mode of action is involved in the inhibition or interruption of the dynamic biosynthesis process of the bacterial cell wall or cytoplasm membrane. These antibiotics are collectively referred to as bacterial cell envelope-acting antibiotics. It is well known that the bacterial cell envelope is indispensable for the survival of bacteria (Silhavy et al. 2010). To cope with the cell envelope stress elicited by these antibiotics, bacterial cells evolve several regulatory systems to regulate gene expression involved in the production of specific resistance determinants, including the extracytoplasmic function (ECF) σ factors-mediated regulatory systems and the two-component regulatory systems (TCSs) (Helmann 2016; Jordan et al. 2008; Mitchell and Silhavy 2019).

The ECF σ factors-mediated regulatory systems are present in both Gram-positive bacteria and Gram-negative bacteria (Helmann 2002; Lonetto et al. 2019). In *Bacillus subtilis*, seven ECF σ factors, such as σ^W and σ^M, are encoded to regulate gene expression by sensing different stimuli. It has been reported that σ^M is activated by the antibiotics targeting bacterial cell envelope (Horsburgh and Moir 1999; Kingston et al. 2013; Thackray and Moir 2003). The *sigM* operon is composed of the σ^M factor coding gene *sigM*, the anti-σ^M factor coding gene *yhdL*, and the anti-σ^M factor accessory protein-coding gene *yhdK*. Under normal conditions, YhdL and YhdK are capable of forming the anti-σ^M factor complex YhdLK, leading to the inhibition of σ^M activity. However, the σ^M is released from the anti-σ^M factor complex under cell envelope stress conditions, and then σ^M activates the expression of its target genes (Asai 2018; Zhao et al. 2019).

At present, several whole-cell biosensors based on transcriptional regulators or two-component systems have been constructed for the screening of new antibiotics (Rebets et al. 2018; Urban et al. 2007; Wolf and Mascher. 2016). For example, the BlaR1/BlaI regulatory system and the two-component system LiaRS were employed to construct biosensors for the screening of β-lactam antibiotics and cell wall active antibacterial compounds, respectively (Kobras et al. 2017; Lautenschläger et al. 2020; Wolf and Mascher 2016). However, there are few studies involved in the construction of biosensors based on the ECF σ factors-mediated regulatory systems and the detection of cell envelope-acting antibiotics. In this study, we develop a whole-cell biosensor based on the σ^M-mediated regulatory system in *B. subtilis* for the detection of antibiotics that target bacterial cell envelope.

Materials and methods

Bacterial strains and culture conditions

Escherichia coli DH5α (DSM 6897) was used as the cloning host. *B. subtilis* subsp. subtilis str. 168 (BGSC 1A1) was used as a host for construction and characterization of whole-cell biosensors. The polymyxin producer, *Paenibacillus polymyxa* C12, was provided by Zhejiang Qianjiang Biochemical Co., Ltd., Haining, China. Bacterial strains were cultivated in Luria–Bertani (LB) broth. For solid agar plates, 1.5% (w/v) agar was added to the medium. For *B. subtilis* transformation, Cells were grown in a Spizizen medium (GMI and GMII). All experiments were performed at 37 °C unless otherwise noted. When needed, the medium was supplemented with chemicals at the following concentrations: ampicillin (Amp), 100 μg/mL; kanamycin (Km), 50 μg/mL; chloramphenicol (Cm), 25 μg/mL.

Construction of the biosensors

For the construction of the Biosensor 1 strain, the fragment of the *ypuA* promoter (P_{ypuA}) was amplified from the genome of *B. subtilis* 168 (Genbank: NC_000964.3). The plasmid pWBUC02 (Yin et al. 2019) was linearized by reverse PCR. Then, the P_{ypuA} fragment was ligated with the linearized plasmid skeleton pWBUC02 by One-Step Cloning Kit (Vazyme), resulting in the recombinant plasmid pWBUC02-P_{ypuA}-*egfp*. The correct recombinant plasmid was extracted by the AxyPrep™ Plasmid Miniprep kit (Axygen) and transformed into *Bacillus subtilis* 168 by the Spizizen's method (Anagnostopoulos and Spizizen 1961), and the final transformant was screened by kanamycin (10 μg/mL) to obtain the correct biosensor strain.

The Biosensor 2 was constructed by introducing a recombinant plasmid pHCMC04-P_{xylA}-*sigM*-P_{43}-*yhdLK* into Biosensor 1. The pHCMC04-P_{xylA}-*sigM*-P_{43}-*yhdLK* was constructed by restriction enzyme cloning and one-step cloning. Firstly, the fragments of the P_{43} promoter from the plasmid pWBUC02 and *yhdLK* (GenBank: NP_388832-NP_388831) were amplified and fused by PCR. The linearized fragment of pHCMC04 (BGSC ECE189P) (Nguyen et al. 2005) was obtained by reverse PCR, and then the fusion fragment was ligated into the linearized plasmid by one-step cloning as described above. The recombinant plasmid pHCMC04-P_{43}-*yhdLK* was obtained by the transformation in *E. coli* DH5α. The *sigM* (GenBank: NP_388833) was inserted as a *Spe*I/*BamH*I digestion in pHCMC04-P_{43}-*yhdLK* downstream of the P_{xylA} promoter. The final correct plasmid was transformed into Biosensor 1 by the Spizizen's method, resulting in Biosensor 2 strain.

All sequences and primers used in this study are shown in Table S1 and Table S2, respectively.

Determination of the relative fluorescence units

The whole-cell biosensor strains were grown overnight in LB broth. The overnight bacterial cultures were sub-cultured 1:100 in 50 mL fresh LB broth and grown to an OD_{600} of

0.2. An aliquot of 3 mL bacterial culture was mixed with varying concentrations of polymyxin B (0, 0.03125, 0.0625, 0.125, 0.25, 0.5, 1, 2, 4, 8, 12, and 16 μg/mL), polymyxin E (0, 4, and 8 μg/mL), D-cycloserine (0, 4, and 8 μg/mL), bacitracin (0, 25, and 50 μg/mL), ampicillin (0, 2, and 4 μg/mL), fosfomycin (0, 1, and 2 μg/mL), kanamycin (0, 0.5, and 1 μg/mL), chloramphenicol (0, 0.25, and 0.5 μg/mL), and lysozyme (0, 5, and 10 μg/mL). After incubation for 4 h at 37 ℃ with 180 rpm shaking, 200 μL cell cultures were transferred to 96-well microplates and 96-well black microplates for measurements of absorbance and fluorescence, respectively. The absorbance was measured at 600 nm, and the fluorescence was measured with excitation and emission wavelengths at 395 and 509 nm, respectively. The displayed results are the relative fluorescence units normalized by OD_{600} (normalized RFU).

Plate-based bioassay

A double-layered plate method was employed to determine the response of the biosensor as described previously with minor modification (Kobras et al. 2017). The whole-cell biosensor strains were grown overnight in LB broth. The overnight bacterial cultures were sub-cultured 1:100 in 50 mL fresh LB broth and grown to an OD_{600} of 0.2. The resulting cell cultures were mixed with the soft MH agar (1:10, v/v) and then poured the entire mixture onto an MH agar plate and swirl gently. After the plate was cooled, 6-mm filter paper was placed on the agar surface. Various concentrations of polymyxin B (0, 0.3125, 0.625, 1.25, 2.5, 5, and 10 mg/mL), D-cycloserine (10 mg/mL), bacitracin (10 mg/mL), ampicillin (100 μg/mL), fosfomycin (10 mg/mL), chloramphenicol (100 μg/mL), and lysozyme (10 mg/mL) were dropped on the filter paper. After incubation for 20 h at 37 °C, the fluorescence of the agar plates was captured on an iBright 1500 Imaging System (Thermo Fisher Scientific).

Detection of polymyxins produced by its producing bacteria

The production of polymyxins by *P. polymyxa* C12 was measured in both liquid broth and agar plates. The whole-cell biosensor strains were grown overnight in LB broth. The overnight bacterial cultures were sub-cultured 1:100 in 50 mL fresh LB broth and grown to an OD_{600} of 0.2. For determination in liquid broth, an aliquot of 3 mL bacterial culture was mixed with 1 mL cell-free culture supernatant of *P. polymyxa* C12. At the same time, 5 μg/mL PMB was used as a positive control. After incubation for 4 h at 37 °C with 180 rpm shaking, the absorbance and the fluorescence were measured, respectively. The displayed results are the relative fluorescence units normalized by OD_{600} (normalized RFU). For determination on agar surfaces, the double-layered plate containing the biosensor strain was prepared and 10 μL cell-free culture supernatant of *P. polymyxa* C12 was added to the filter paper. *B. subtilis* 168 strain was used as a negative control. After 20 h of culture at 37 °C, the fluorescence of the agar plates was captured on an iBright 1500 Imaging System (Thermo Fisher Scientific).

Screening for bacteria producing cell envelope-acting antibiotics from soil

Six *Bacillus* strains isolated from soil were grown in LB broth for 2 days. The double-layered plate containing Biosensor 1 was prepared and 10 μL cell-free culture supernatant of these *Bacillus* strains was added to the filter paper. After 20 h of culture at 37 °C, the fluorescence of the agar plates was captured on an iBright 1500 Imaging System (Thermo Fisher Scientific).

Data analysis

All experiments were performed in biological triplicates and the standard deviations of the three values were calculated. Experimental values are presented as the mean ± standard error of the mean (SEM). The Student's *t*-test was used to compare statistical differences between the groups of experiment data. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

Results

Construction of a whole-cell biosensor for detection of cell envelope-acting antibiotics

It has been reported that the extracytoplasmic function factor σ^M in *Bacillus subtilis* monitors cell envelope stress, which is commonly induced by various cell envelope-acting antibiotics (Thackray and Moir 2003). In the presence of these antibiotics, the σ^M is released from the anti-σ^M factor, and then σ^M regulates the transcription of its target genes (Zhao et al. 2019) (Fig. 1A). We reasoned that the σ^M-mediated regulatory system can be used to construct a whole-cell biosensor for the detection of antibiotics targeting bacterial cell envelope. To this end, several σ^M-regulated promoters, including P_{ypuA}, P_{ypbG}, P_{yacK}, P_{ydaH}, and P_{sigM}, were fused with the reporter gene *egfp* (encoding enhanced green fluorescent protein), and then transformed into *Bacillus subtilis* 168 strain. Upon treatment with polymyxin B (PMB), a cationic antimicrobial peptide that targets the cell envelope, the relative fluorescence units (RFU) of all these resulting strains were increased compared with the untreated control group (Fig. 1B). Among them, the P_{ypuA} had the most significant changes in the output signal. After PMB was added at a concentration of 4 μg/mL and 8 μg/mL, the fluorescence signals

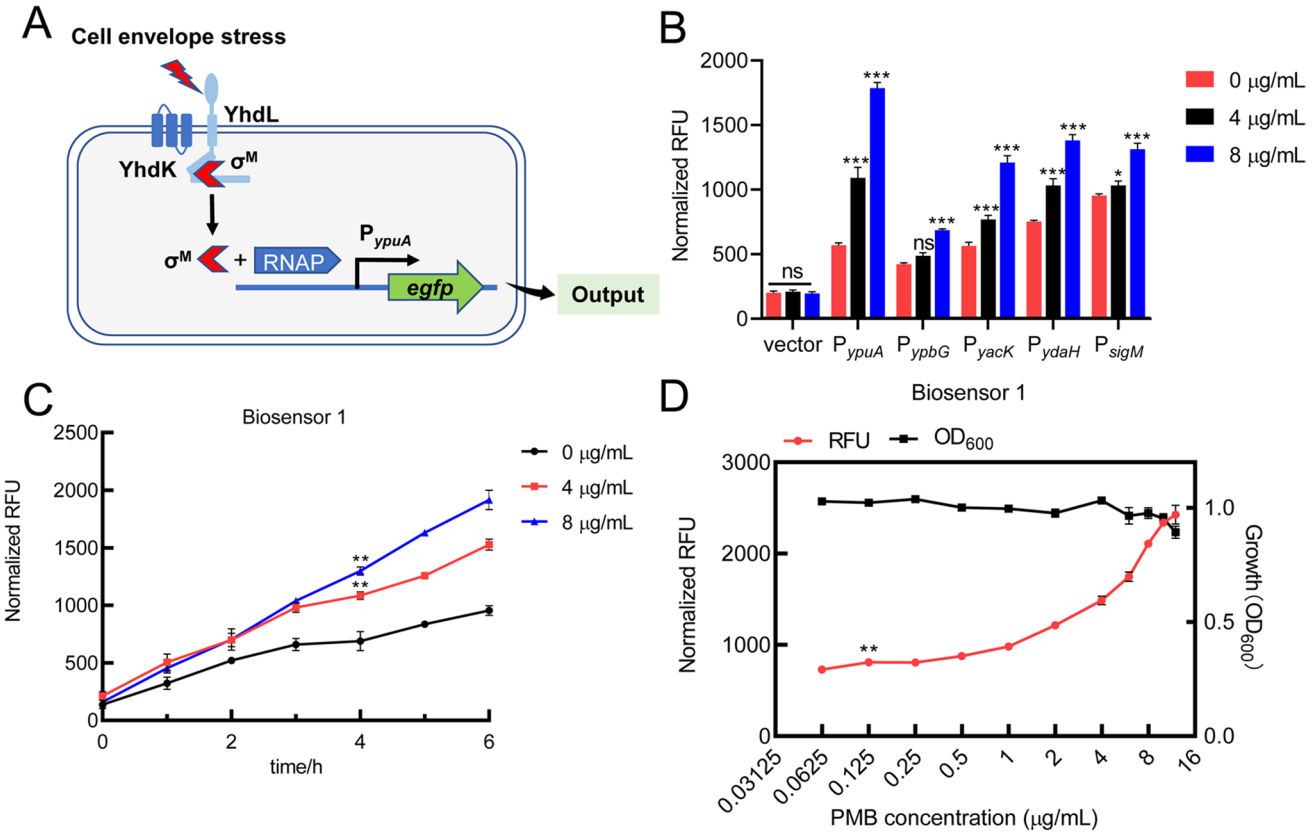

Fig. 1 Construction of a whole-cell biosensor for PMB detection. **A** Schematic illustration of genetic circuit of Biosensor 1. **B** Screening for the promoter in response to PMB. The *x*-axis indicates different σ^M-regulated promoters, which fused with *egfp*. The vector represents the *egfp* gene without promoter. The *y*-axis shows the relative fluorescence units normalized over OD_{600} (normalized RFU). The figure demonstrates the fluorescence output post-induction with PMB at 4 h. **C** The response time of the biosensor in presence of PMB at different concentrations. **D** Dose responses of the biosensor. After incubation with PMB at 4 h, growth of Biosensor 1 was measured as OD_{600}, and then the fluorescence output was calculated

driven by the P_{ypuA} were 1.9-fold and 3.1-fold stronger than that without PMB addition, respectively. Therefore, the P_{ypuA} was chosen for further studies. The whole-cell biosensor based on the P_{ypuA} was designated Biosensor 1.

Next, the time-dependent responses of Biosensor 1 were measured for 6 h after adding 4 μg/mL and 8 μg/mL PMB. As shown in Fig. 1C, the fluorescence signal with PMB at a concentration of either 4 or 8 μg/mL was higher than the background signal under all tested conditions. After incubation for 4 h, the fluorescence signals were increased by about 2.0 times ($P < 0.01$) in the presence of 4 μg/mL and 8 μg/mL PMB. The dose-dependent responses of Biosensor 1 were also determined upon exposure to PMB at 0 to 16 μg/mL for 4 h. As shown in Fig. 1D, the fluorescence intensity for Biosensor 1 was positively correlated with the concentrations of PMB. The fluorescence signal was noticeable when PMB was added at a concentration as low as 0.125 μg/mL ($P < 0.01$). When PMB was added at concentrations higher than 12 μg/mL, growth of Biosensor 1 was dramatically inhibited, which will affect the biosensor performance. Therefore, the concentration range of PMB that can be detected by Biosensor 1 was between 0.125 and 12 μg/mL.

Specificity of the biosensor

To explore the specificity of Biosensor 1, we detected the fluorescence signals after exposure to various antibiotics, including polymyxin E, D-cycloserine, bacitracin, ampicillin, fosfomycin, kanamycin, and chloramphenicol. Among them, the first five antibiotics target cell envelope. While polymyxin E is structurally and functionally related to PMB (Yu et al. 2015), the other four antibiotics inhibit cell wall biosynthesis at different steps (Kohanski et al. 2010). However, kanamycin and chloramphenicol interrupt the process of protein synthesis (Kohanski et al. 2010). Sub-inhibitory concentrations (Sub-MIC) of antibiotics were selected since high levels of antibiotics significantly inhibit bacterial cell growth. As shown in Fig. 2A ~ E, the addition of all antibiotics that target the cell envelope

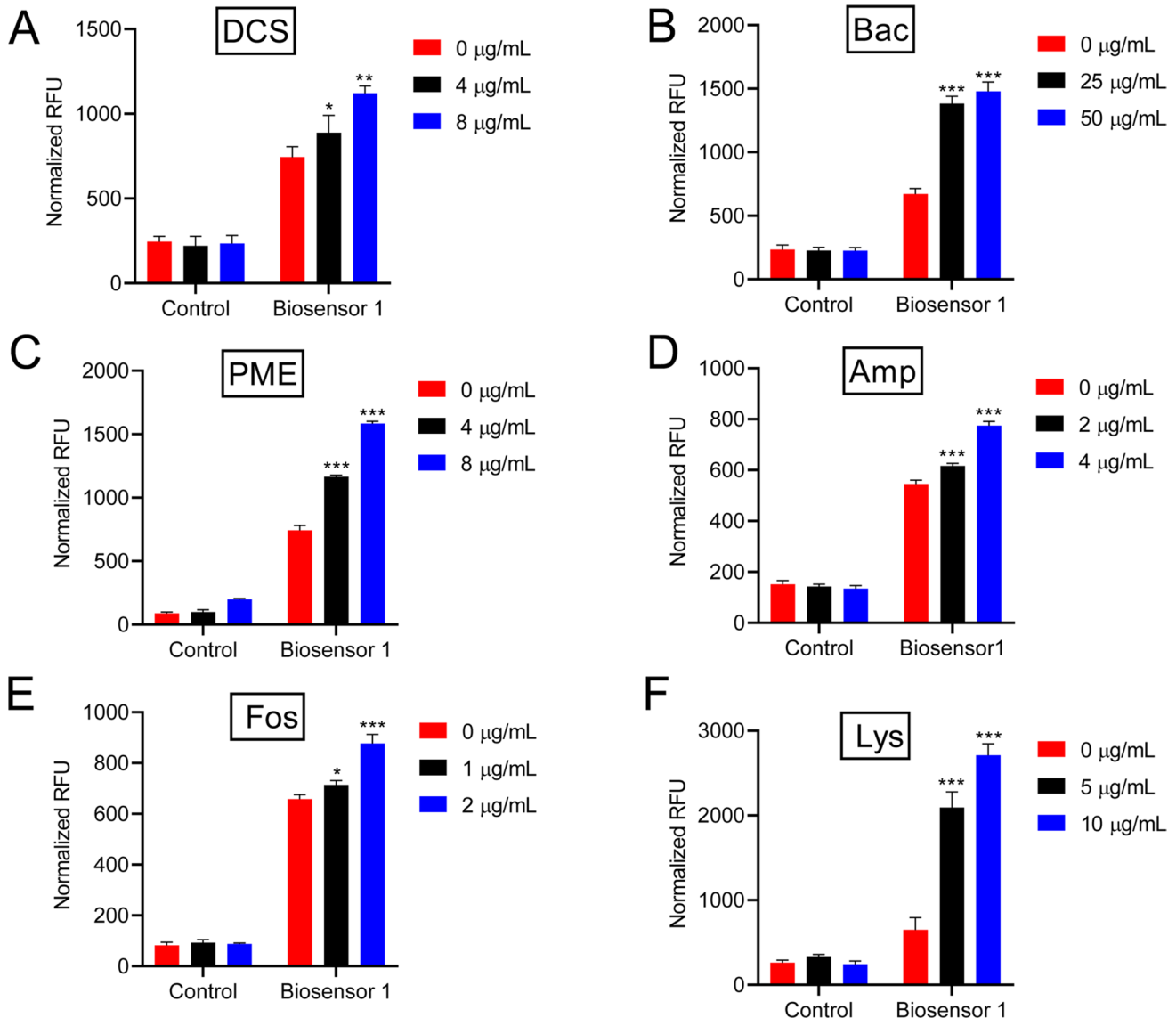

Fig. 2 The response of Biosensor 1 in the presence of D-cycloserine (DCS) (**A**), bacitracin (Bac) (**B**), polymyxin E (PME) (**C**), ampicillin (AMP) (**D**), fosfomycin (FOS) (**E**), and lysozyme (Lys) (**F**). The control represents *B. subtilis* 168 harboring the *egfp* gene without promoter. The biosensors were treated with sub-MIC concentrations of antibiotics at 4 h, and then the relative fluorescence units normalized over OD_{600} (normalized RFU) were calculated

increased the fluorescence signals in a concentration-dependent manner. In contrast, kanamycin and chloramphenicol failed to increase the fluorescence signal of the biosensor (Fig. S1). In addition, we also determined the response of the biosensor in the presence of lysozyme, which is active against Gram-positive bacteria by hydrolyzing cell wall peptidoglycan (Ragland and Criss 2017). The results showed that the fluorescence signals were significantly increased when treated by lysozyme (Fig. 2F). In conclusion, these results suggest that the response of Biosensor 1 is specific to bacterial cell envelope-acting antibiotics.

Controlled expression of anti-σ^M and σ^M reduced biosensor background

Thus far, Biosensor 1 constructed by the σ^M-mediated regulatory system is capable of detecting cell envelope-acting antibiotics. However, we noticed that this biosensor displayed a high background signal in the absence of antibiotics. To reduce the background fluorescence signal, the expression of the anti-σ^M and σ^M was optimized in Biosensor 1. The expression of the anti-σ^M factor YhdL and YhdK (encoding by *yhdLK*) was driven by the strong constitutive promoter P_{43}, while the expression of the σ^M was under the

control of the xylose-inducible promoter P_{xylA}. The resulting whole-cell biosensor strain was named Biosensor 2. Similar to Biosensor 1, the fluorescence signal of Biosensor 2 was positively correlated with the concentrations of PMB, regardless of the level of xylose (Fig. 3). Notably, the background fluorescence signal of Biosensor 2 was significantly reduced in the presence of xylose at low concentrations (less than 1%) when compared with Biosensor 1. After xylose was added at 1%, the background fluorescence signal was reduced by 23%. Besides, the fluorescence signals induced by PMB in Biosensor 2 were also lower than that in Biosensor 1. Therefore, the controlled expression of anti-σ^M and σ^M can reduce the background fluorescence signal of the biosensor.

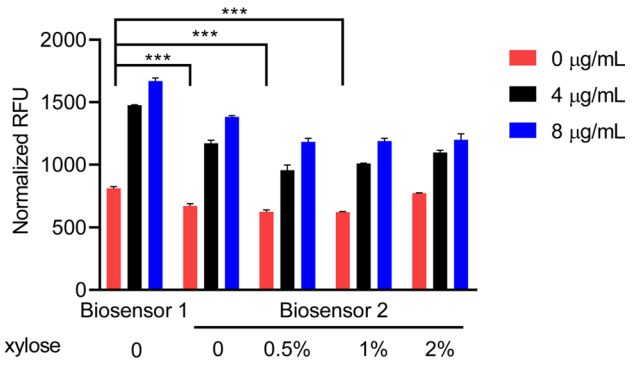

Fig. 3 The response of Biosensor 2 in the presence of PMB at different concentrations. In Biosensor 2, the expression of anti-σ^M and σ^M were under the control of the P_{43} promoter and xylose-inducible promoter P_{xylA}, respectively

Plate-based bioassay for the biosensor

After measuring the response of the biosensors in liquid cultures, we proceeded to determine the response of the biosensors on agar plates using a simple "spot-on-lawn" assay (Kobras et al. 2017). At first, we investigated the fluorescence signal of Biosensor 1 in the presence of PMB from 0 to 10 mg/mL. As shown in Fig. 4A, the induction of Biosensor 1 was not observed in the absence and presence of low levels of PMB (< 0.625 mg/mL) after incubation for 20 h. However, when PMB was added at concentrations higher than 1.25 mg/mL, Biosensor 1 yielded a ring-shaped

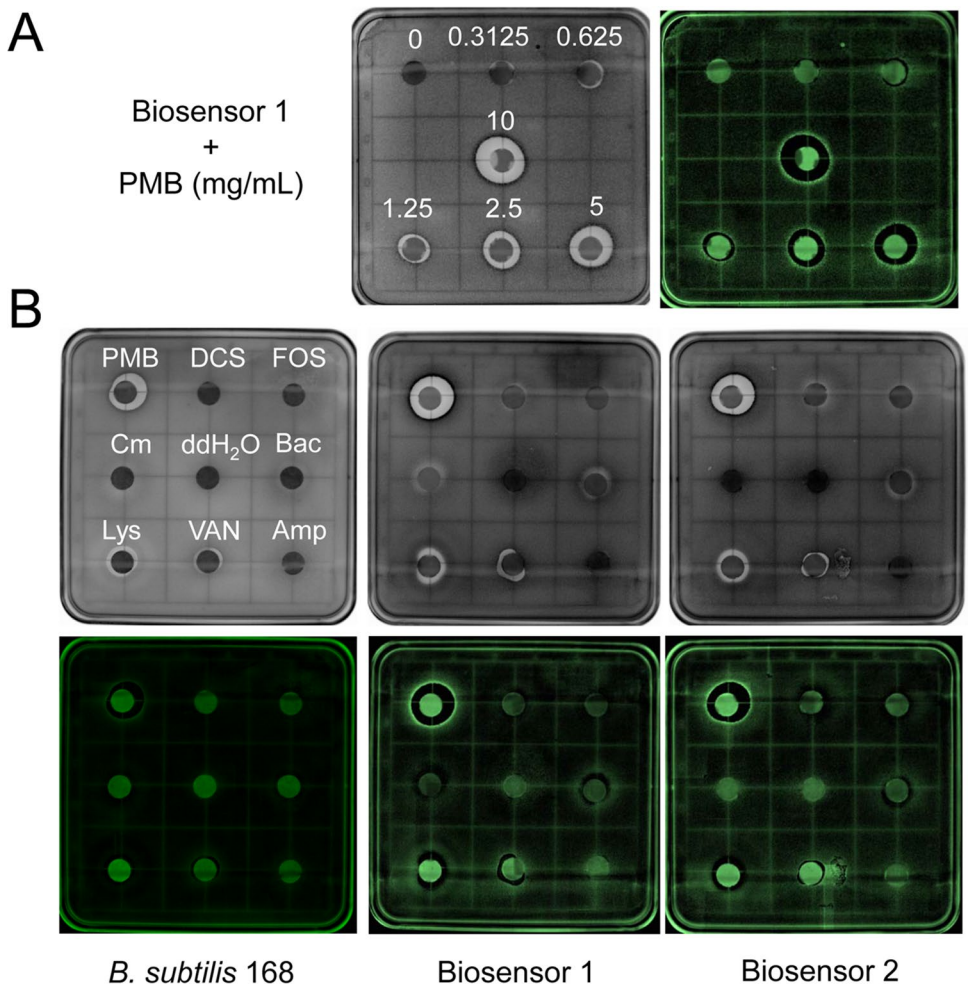

Fig. 4 Detection of cell envelope-acting antibiotics by plate-based bioassay. **A** Detection of different concentrations of PMB by Biosensor 1. White light image (left) indicates the positions of the disks on the plate. The corresponding image from fluorescence detection is displayed right. **B** Detection of different antibiotics by the two biosensors and the control (*B. subtilis* 168). The upper row shows the white light images, while the corresponding fluorescence images are displayed underneath. PMB, polymyxin B; DCS, D-cycloserine; FOS, fosfomycin; Cm, chloramphenicol; Bac, bacitracin; Lys, lysozyme; VAN, vancomyxin; AMP, ampicillin

fluorescence signal surrounding the filter paper. In agreement with the results from the liquid cultures, the fluorescence intensity of Biosensor 1 on agar surfaces was also positively correlated with the concentrations of PMB. We next determined the fluorescence signal of Biosensor 1 and Biosensor 2 upon exposure to various cell envelope-acting antibiotics on agar surfaces. Results showed that both biosensors are capable of detecting several of these antibiotics, including PMB, bacitracin, vancomycin, and lysozyme but not chloramphenicol, whereas the negative control strain *B. subtilis* 168 cannot detect any antibiotics (Fig. 4B).

Detection of polymyxin production by *Paenibacillus polymyxa*

Polymyxins are produced by the Gram-positive bacterium *Paenibacillus polymyxa* C12 (Yu et al. 2019). To explore whether our biosensors can detect polymyxin from cultures of the producer strain, we collected the cell-free culture supernatant of *P. polymyxa* C12 and then subjected it to biosensor performance assays both in liquid cultures and on agar plates. In liquid cultures, both Biosensor 1 and Biosensor 2 exhibited about a fourfold increase in fluorescence signal when supplemented with the cell-free culture supernatant of *P. polymyxa* C12 (Fig. 5A). Notably, the fluorescence signal for the two biosensors was much higher than that induced by 5 μg/mL PMB. On agar plates, the addition of both the cell-free culture supernatant and the cultures of *P. polymyxa* C12 resulted in a ring-shaped fluorescence signal of Biosensor 1 (Fig. 5B), which is consistent with the data in liquid cultures. Interestingly, the response of the biosensor induced by the cell-free culture supernatant was stronger than that induced by the cultures of *P. polymyxa* C12. In comparison, *B. subtilis* 168 did not affect the response of Biosensor 1. Therefore, these results suggest that our biosensors can be used for the detection of not only PMB but also PMB-producing bacterium.

Biosensor-guided identification of bacteria producing cell envelope-acting antibiotics

Next, we used Biosensor 1 to identify bacteria that produce cell envelope-acting antibiotics from environmental samples. To this end, the cell-free culture supernatants of six *Bacillus* strains isolated from soil were dropped onto the agar plates containing Biosensor 1 (Fig. 6). *Bacillus* strains were used in this study given that the genus *Bacillus* can produce a large number of antimicrobial compounds (Caulier et al. 2019). After incubation for 20 h, a ring-shaped fluorescence signal was observed for one *Bacillus* isolate (B3), an indicator of the production of cell envelope-acting antibiotics. Comparison of the bacterial 16S rRNA gene sequence showed that the isolate B3 shares 99% identities with several members of *Bacillus velezensis* and *Bacillus amyloliquefaciens*. These

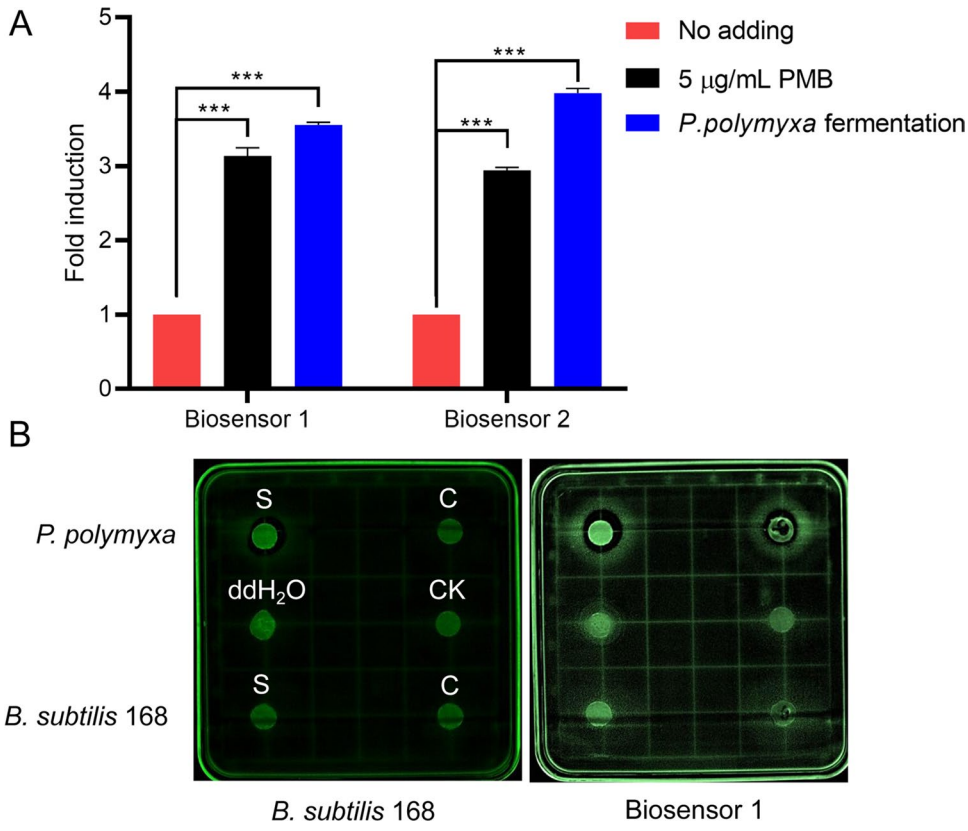

Fig. 5 Detection of polymyxin produced by *Paenibacillus polymyxa* C12 in both liquid cultures (**A**) and agar surfaces (**B**). In liquid cultures, fold induction of the normalized RFU relative to an untreated control is indicated. For plate-based bioassay, bacterial cells were grown in LB broth for 2 days. Then, the cell-free culture supernatant (S) and culture (C) of each bacteria were subjected to biosensor performance assay

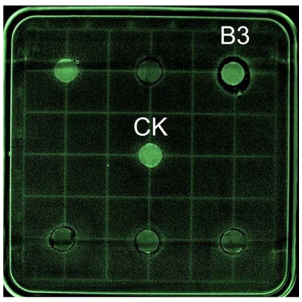

Fig. 6 Screening for bacteria producing cell envelope-acting antibiotics. Six *Bacillus* strains isolated from soil were grown in LB broth for 2 days. The cell-free culture supernatant was subjected to biosensor performance assay

results suggest that the σ^M-based biosensors can be used to screen bacteria producing cell envelope-acting antibiotics.

Discussion

There are several regulatory systems in response to bacterial cell envelope stress in both Gram-positive and Gram-negative bacteria (Helmann 2016; Jordan et al. 2008; Mitchell and Silhavy 2019). Several whole-cell biosensors have been constructed based on the two-component systems; however, very few attempts have been made to construct biosensors by using the ECF σ factors-mediated regulatory systems. In this study, we used the σ^M-mediated regulatory system of *B. subtilis* to design a whole-cell biosensor for the detection of cell envelope-acting antibiotics.

Using polymyxin B as the bacterial cell envelope stress and enhanced green fluorescent protein as the reporter, we found that the P_{ypuA} had the lowest background noise and the most significant changes in the fluorescence output. The whole-cell biosensor displayed dose-dependent and time-dependent responses in fluorescence signals. The detection range of polymyxin B by this biosensor was between 0.125 and 12 μg/mL. The response of the biosensor is specific to antibiotics that target cell envelope. Besides determination in liquid broth, the fluorescence output of the biosensor can be easily determined on agar surfaces. Using this biosensor, we successfully detected polymyxins secreted by the polymyxin-producing strain and bacteria that produce cell envelope-acting antibiotics.

Given that the σ^M factor is capable of sensing bacterial cell envelope stress, the biosensor constructed in this study has certain advantages in the discovery of antimicrobial compounds. It can be used to identify antibiotics targeting not only cell membrane but also cell wall in contrast to the fact that several previous biosensors can only detect antibiotics with one target. For example, biosensors based on the two-component systems LiaRS can detect cell wall active antibiotics (Kobras et al. 2017; Wolf and Mascher 2016), while a biosensor based on BlaR1/BlaI could sense β-lactam antibiotics (Lautenschläger et al. 2020). Moreover, the biosensor constructed by σ^W, another ECF σ factor in *B. subtilis*, is responsive to the antibiotics targeting cell wall, but not to the antibiotics targeting cell membrane (Czarny et al. 2014). Therefore, compared with that, our σ^M-based whole-cell biosensor can detect more antibiotics from the environments, which is more suitable for the initial screening of novel antibiotics.

Additionally, our results showed that the σ^M-based whole-cell biosensor is responsive to the bacterial cell cultures of polymyxin-producing *P. polymyxa* C12. Thus, this biosensor could also be used in several aspects in industrial microbiology. The first one is to develop a high-throughput screening method for the identification of bacteria with high productivity of cell envelope-acting antibiotics. It will be an easy task to assess the yield of antibiotics from a bacterial library. Another one is to online monitor the concentration of a certain cell envelope-acting antibiotic in bacterial cell cultures. Several whole-cell biosensors for intracellular metabolites, such as lactate (Goers et al. 2017), shikimic acid (Li et al. 2017), and naringenin (Skjoedt et al. 2016), have been designed for monitoring their concentrations in cell cultures.

At present, most whole-cell biosensors are designed based on transcription factors. The properties of these kinds of biosensors can be improved by protein engineering of the transcription factors, which alters the effector binding specificity (Cheng et al. 2018; Kasey et al. 2018). However, the σ^M factor cannot directly bind to effector molecules, but senses cell envelope stress by anti-σ^M factor. Therefore, it might be impossible to improve the properties of the σ^M-based biosensor by protein engineering. Instead, we optimized the expression of the anti-σ^M and σ^M in the biosensor. The resulting new biosensor (Biosensor 2) exhibited a reduced background fluorescence signal. Meanwhile, the response of Biosensor 2 was also reduced in the presence of antibiotics, thus restricting the further application of Biosensor 2. Recent studies showed that the stress-responsive promoters in *B. subtilis* can be enhanced by interlocking the binding motifs of several σ factors (Wang et al. 2019a; 2019b). The effect of this strategy on the response of the σ^M-based biosensor needs to be further investigated.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00253-022-11762-z.

Author contribution JY, DC, and ZY conceived and designed research. DC, YZ, and YL conducted experiments. JY, DC, and ZY analyzed data. JY, DC, and ZY wrote the manuscript. All authors read and approved the manuscript.

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Data availability All data generated or analyzed during this study are included in this article (and its supplementary information files).

Declarations

Ethics approval This article did not contain research involving humans or animals performed by any of the authors.

Conflict of interest The authors declare no competing interests.

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