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Reprogramming Probiotic *Lactobacillus reuteri* as a Biosensor for *Staphylococcus aureus* Derived AIP-I Detection

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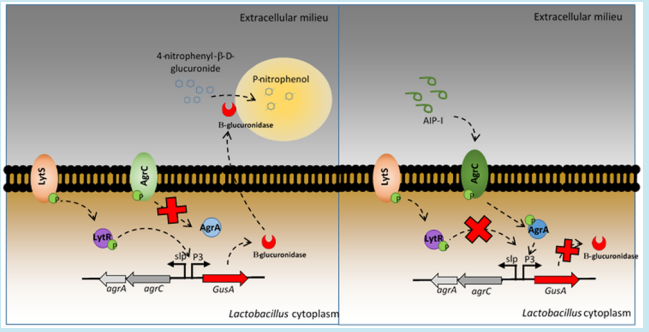
Supporting Information

ABSTRACT: Gram-positive *Staphylococcus aureus* infection that results in pneumonia, urinary tract infection, and in severe cases, sepsis, has recently been classified as a serious threat to public health. Rapid and cost-effective detection of these infections are costly and time-consuming. Here, we present probiotic lactic acid bacteria engineered to detect autoinducer peptide-I (AIP-I), a quorum sensing molecule produced by *Staphylococcus* sp. during pathogenesis. We achieved this by adapting the well-characterized *agr* quorum sensing (*agr*QS) from *Staphylococcus aureus* into *Lactobacillus reuteri*. The engineered biosensor is able to detect AIP-I levels in the nanomolar to micromolar range. We further investigated the function of the biosensor to detect real-time changes in AIP-I levels to understand the dynamics of *Staphylococcus aureus* under various strenuous conditions. The developed sensors would be useful for detection of *Staphylococcus* contamination in hospital settings and for high-throughput drug screening.



KEYWORDS: *whole cell biosensor, Staphylococcus aureus, quorum sensing, Lactobacillus reuteri*

Health care-associated or nosocomial infections are a rising problem in hospitals that affect primarily immune compromised patients exposed to aseptic environments of the hospital.[1] These nosocomial infections, which infect 7 to 10% of hospitalized patients, spread easily from patient to patient sharing common space (i.e., wards, beds, seats, toilets) and through the use of aseptic medical instruments.[1,2] Early detection of such infections and the source of the contamination allows early treatment of patients, resulting in higher patient survival rates. Unfortunately, most methods of diagnosis involve culturing and staining of patient samples, multiplex PCR, biochemical assays, and hemodynamic studies, which are time-consuming and/or costly.[3] Thus, a rapid detection of microbial infection and accurate diagnosis would enable healthcare officers to administer effective treatment in a timely manner, further preventing the infection from spreading.

In recent years, medical diagnostics and therapy have adopted a synthetic biology approach to engineer microbes for therapeutic applications. The reprogrammed microbes have the ability to treat diseases,[4] deliver therapeutics,[5] tackle cancer,[6,7] and fight pathogens.[8] The hallmark of using cell-based therapeutics is the introduction of a sense and respond mechanism that is exogenous to the host cell. Examples include engineered microbes such as *E. coli* that respond to *Pseudomonas aeruginosa* quorum sensing molecules,[8] mimick cyanobacterial circadian clocks,[9] or respond to metabolites present in the environment.[10−12] In tackling nosocomial infections, pathogen quorum-sensing molecules (chemical compounds involved in microbial cross talk) are used for the detection of pathogenic infections and determination of the virulence state of the infection. In previous studies, microbes have been engineered to respond to quorum sensing molecules from Gram-negative pathogens such as *P. aeruginosa*,[8] *E. faecalis*,[13] and recently *V. cholera*.[14] However, there have been limited studies on using microbes to sense quorum-sensing molecules from Gram-positive pathogens. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a multi-antibiotic resistant Gram-positive *Staphylococci*[15] associated with severe skin infection in medical settings that can lead to sepsis and death.[16] Over the last years, the frequency of MRSA-related deaths surpassed those caused by HIV and is classified by the Centre for Disease Control (CDC) as a serious public health threat.[17] The Gram-positive *S. aureus* uses a peptide-based two-component quorum-sensing system, known as Agr quorum sensing (*agr*QS) to regulate the expression of many virulence genes and biofilm formation, which is crucial for

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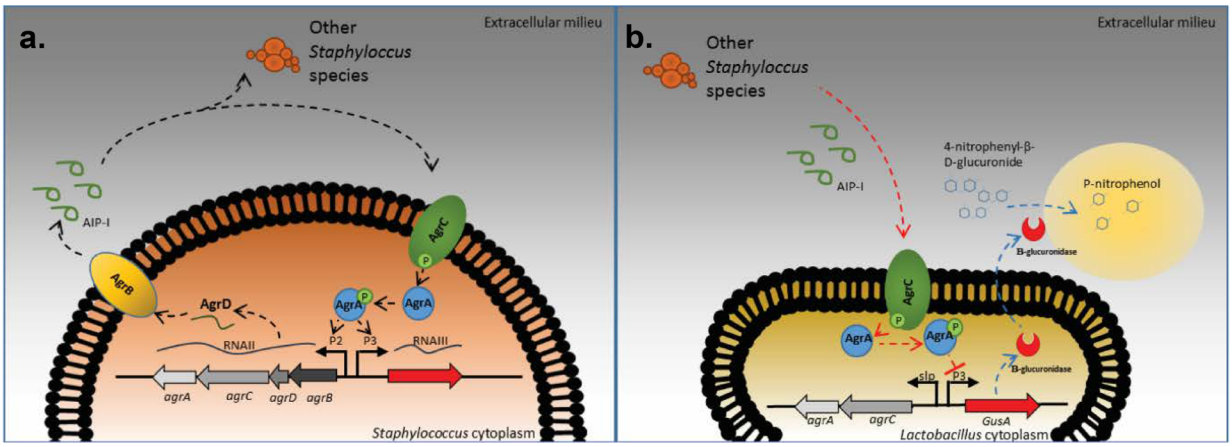

Figure 1. Agr quorum sensing (*agr*QS) in native *Staphylococcus sp.* and engineered *Lactobacillus reuteri* DSM20016. (a) In *Staphylococcus aureus,* RNAII regulated by P2 promoter expresses the regulatory module (AgrA and AgrC), while AgrB helps in the maturation of pro-peptide AgrD to produce AIP-I. The detection of AIP-I by AgrC activates the phosphorylation of AgrA that turns on expression regulated by P2 and P3. (b) The engineered biosensor employs *Lactobacillus reuteri* DSM20016 by introducing the regulatory module under constitutive promoter slp. The *Lactobacillus* host constitutively expresses *β*-glucuronidase that converts 4-nitrophenyl-*β*-D-glucuronide to yellow pigment *p*-nitrophenol (blue arrows). The sensing of AIP-I from other *Staphylococcus* sp. will inhibit *β*-glucuronidase expression by the interaction of phosphorylated AgrA to the P3 site (red arrows).

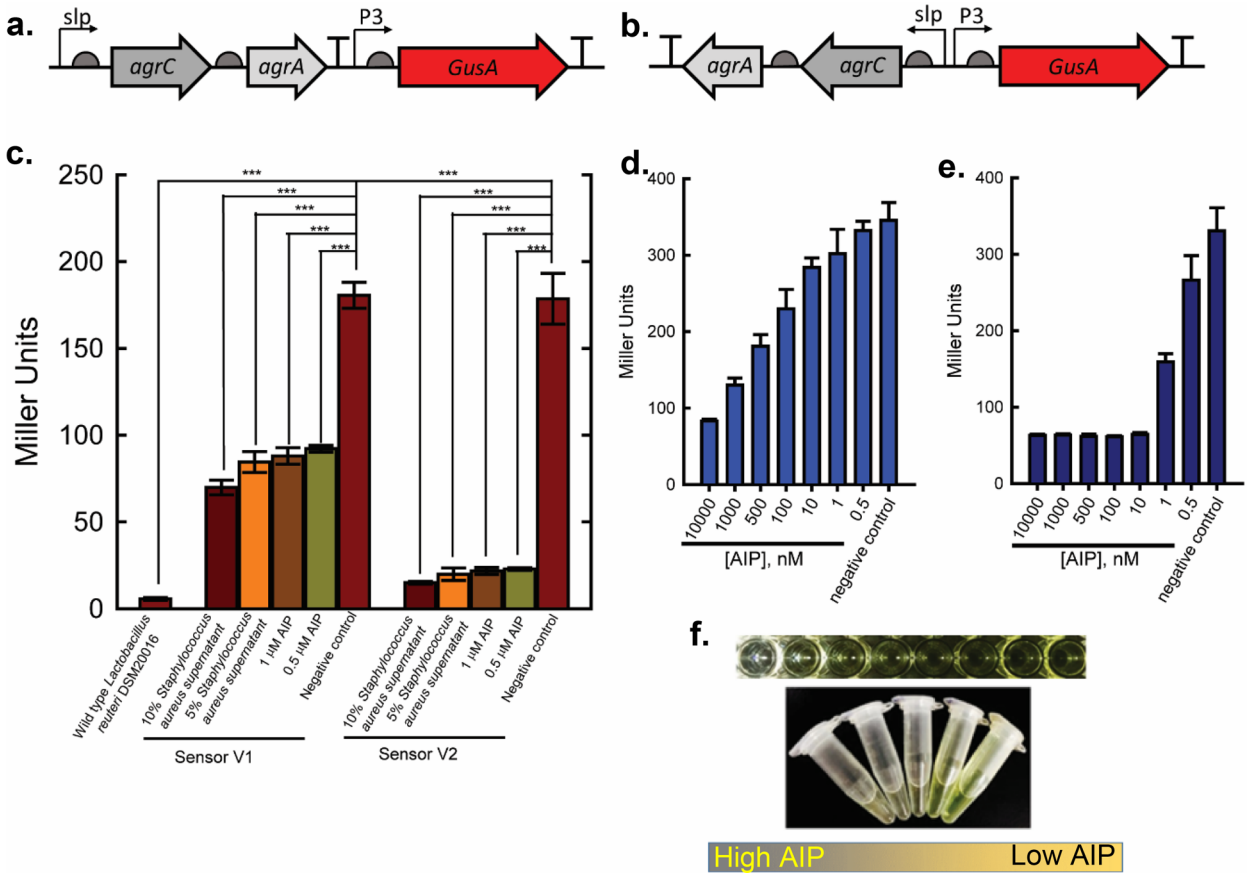

Figure 2. AIP-I biosensor V1 and V2 construct and response to AIP-I. The genetic construct of (a) V1 and (b) V2 biosensor. (c) The response of V1 and V2 to high concentrations of purified AIP-I and *Staphylococcus aureus* supernatant, where NS represents nonsignificant, (∗) $p \leq 0.05$, (∗∗) $p \leq 0.01$, (∗∗∗) $p \leq 0.001$, and error bars represent the standard deviation of biosensor readout using glucuronidase assay. Biosensors (d) V1 and (e) V2 response to increasing concentrations of AIP, where error bars represent the standard deviation of biosensor readout using glucuronidase assay. (f) Colorimetric output of the biosensors challenged with different concentrations of AIP-I.

controlling the microbial motile and sessile lifestyle.[18] This system, regulated by two promoters P2 and P3, encodes for the divergent transcriptional units RNAII and RNAIII, respectively (Figure 1a). RNAII transcript encodes for all four genes *agrB*, *agrD*, *agrC*, and *agrA*, while RNAIII is the intracellular effector molecule that controls the Agr targets, including the expression of virulence factors such as alpha-toxin and delta-hemolysin (Figure 1a).[19] Briefly, AgrD is the pro-peptide that forms the

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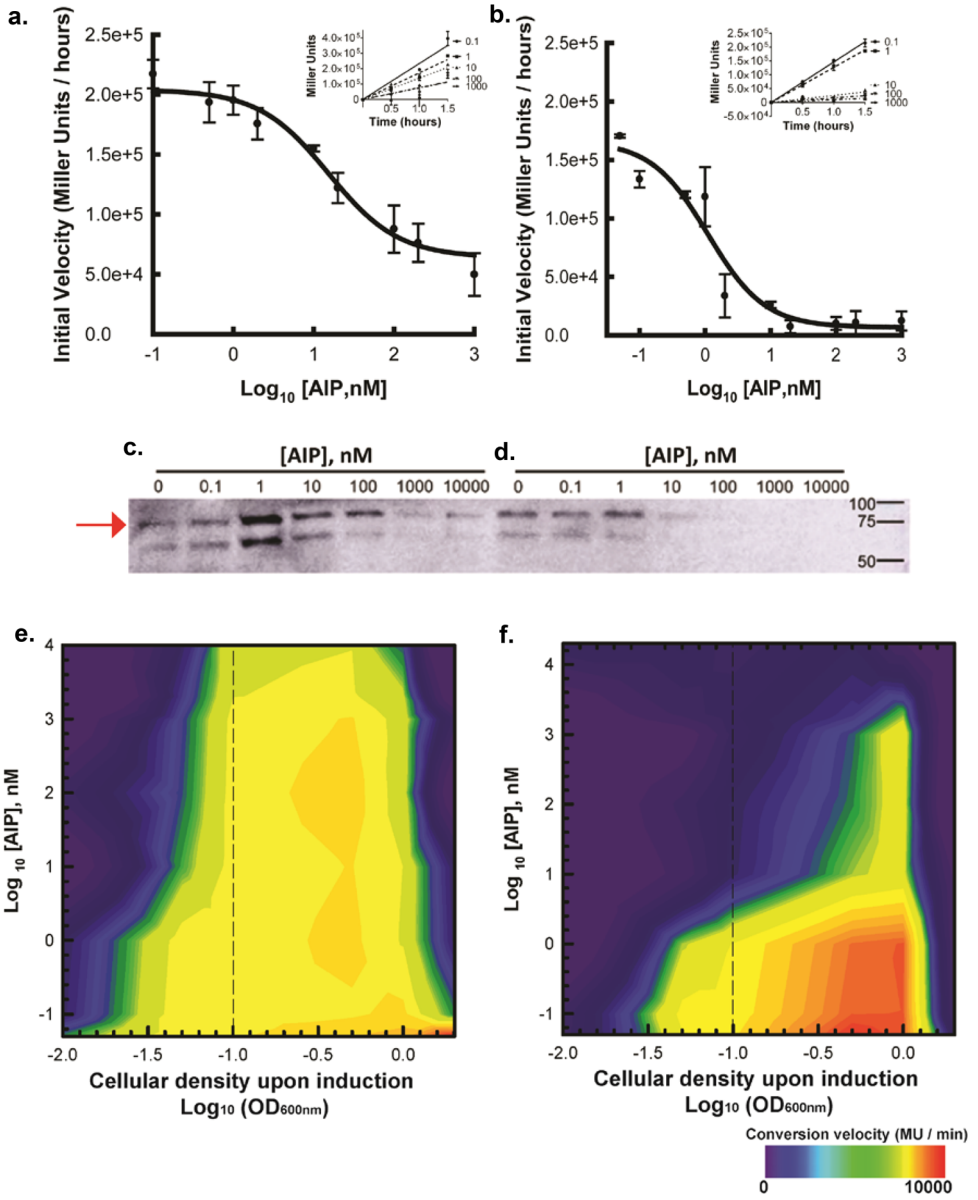

Figure 3. Determination of EC50 of biosensors V1 and V2. AIP-I binding kinetics to reporter (a) V1 and (b) V2 (inset: glucuronidase catalysis of the sensors V1 and V2). (Error bars represents the standard deviation of the glucuronidase catalytic initial velocity when treated with increasing AIP-I concentrations.) Western blot showing the expression of glucuronidase when sensors (c) V1 and (d) V2 were challenged with different concentrations of AIP-I; glucuronidase band (~80 kDa) is annoted by the red arrow. Sensors (e) V1 and (f) V2 heat map indicating the catalytic velocity of sensor cell density when tested at different AIP-I concentrations.

quorum sensing molecule autoinducer peptide (AIP), while AgrB is a transmembrane protein that is responsible for cyclizing AgrD, generating AIP. The remaining members, AgrC and AgrA, form the two-component system responsible for AIP detection and response relay, respectively.[19] Upon binding to AIP, AgrC, a transmembrane histidine kinase receptor, facilitates the phosphorylation of the transcriptional activator AgrA. The phosphorylated AgrA in turn activates gene expression regulated by P2 and P3 promoters producing a positive feedback loop of AIP production and activating various downstream virulence factors.[20] There are four types of AgrC encoded by different *Staphylococcus* (Type I–IV) that specifically detects AIP Type I - IV, respectively.[21]

The aim of this study is to engineer probiotic lactic acid bacteria (LAB) to execute AIP Type-I (AIP-I) *agr*QS behavior for Staphylococcal detection (Figure 1b). AIP-I was selected as our target molecule as it plays an important role in pathogenesis and virulence regulation, particularly during MRSA pathogenesis in infected patients.[19] While *E. coli* may be the preferred chassis for microbial engineering due to the availability of various genetic tools and models, the Gram-negative cell wall composition of *E. coli* is likely ill-suited for integrating the *agr*QS system. Taking into consideration that the host cell should not present any form of potential virulence, especially resulting from interspecies cross talking, we focused on engineering the Gram-positive probiotic LAB, *Lactobacillus reuteri* DSM20016 (27). As an added advantage, *L. reuteri* DSM20016 has been reported to naturally displace and inhibit *S. aureus* proliferation.[22]

Here, we report the engineering of *L. reuteri* as a rapid and high-throughput whole-cell biosensor for the detection of AIP-I. We further report on the adaptability of the *agr*QS system into

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probiotic *L. reuteri* and the observation of an inversed behavior of cells in response to AIP-I.

RESULTS AND DISCUSSION

Circuit Design and Sensor Response to AIP-I. We designed two *agr*QS circuitries in *L. reuteri* (V1 and V2) for sensing AIP-I. By orienting the gene constructs in two different directions, we aimed to toggle the sensitivity of the *agr*QS system, thus developing two sensors with complementing operational ranges.

In the sensor V1, we designed a regulatory module consisting of *agrC* and *agrA* under constitutive expression using a *slp* promoter with native *Staphylococcus aureus* ribosomal binding sites and a double terminator sequence (BBa_B0015) (Figure 2a, Supplementary Figure 1a). We inserted downstream of the regulatory module, the sensing module consisting of the AgrA-activating promoter *P3* that controls the expression of glucuronidase-expressing *GusA* reporter gene similarly flanked by a ribosomal binding site and a double terminator (Supplementary Figure 1b). To evaluate the function of the biosensor, we introduced synthesized AIP-I and *Staphylococcus aureus* cellular lysate to V1 cultures in the early exponential phase. The expression level of glucuronidase was determined using *p*-nitrophenyl *β*-D-glucuronide that hydrolyses in the presence of the enzyme to glucuronic acid (a yellow pigment measurable at $\lambda_{405\ nm}$) (Figure 2f). We observed an inversed behavior in the sensor, in which sensor V1 not given AIP-I expressed glucuronidase constitutively and showed an inhibition of glucuronidase expression when challenged with *S. aureus* physiological concentrations of AIP-I (0.5−1.0 μM) (Figure 2c). Similarly, we observed the same behavior when sensor V1 cultures were incubated with *S. aureus* cell lysate (Figure 2c). However, we noted that the presence of AIP-I at physiological concentrations did not completely inhibit the expression of *GusA,* suggesting leaky *GusA* gene expression that might have resulted from the readthrough effect due to incomplete transcription termination from the upstream regulatory module.

This prompted the development of the second sensor V2 for improved sensitivity to AIP-I concentrations. In the V2 construct, we flipped the orientation of the regulatory module to isolate both modules and prevent any read-through between them (Figure 2b). Similar to V1, *GusA* was expressed constitutively and inhibited with the addition of AIP-I. As anticipated, the V2 sensor responded by complete inhibition of *GusA* expression under physiological concentrations, with a 7-fold lower signal compared to V1 (Figure 2c).

To better understand the capacity of both the biosensors to detect the natural range of AIP-I concentrations in the environment, we proceeded to investigate the sensitivity of both biosensors and their operational range using an increasing concentration of AIP-I. In a previous study conducted by Junio *et al.*, AIP-I isolated from *Staphylococcus aureus* cultured in growth media showed a linear operational range of 2.3 to 63 μM at different phases of growth.[23] We discovered that sensor V1 is able to report in a linear operational range of 10−1000 nM AIP-I (Figure 2d), while sensor V2 is able to report in the sub-nM range (Figure 2e). Thus, the combinatorial use of both sensors presents a rapid and high-throughput assay that can function as both a qualitative (the visible colorimetric means of detection) and quantitative means of detecting AIP-I present in environmental/medical samples.

Detection of AIP-I by AgrC and AgrA in *Staphylococcus aureus* activates the transcription of RNAII and RNAIII which creates a positive feedback that increases AIP-I concentrations.[24] Previously, the *agr* QS system adapted in *Bacillus megaterium* was able to respond to AIP-I similarly to *Staphylococcus aureus* (Supplementary Figure 2a).[25] However, the inverse behavior of the activation of AgrA and AgrC, as well as the constitutive expression controlled by promoter P3 in *Lactobacillus reuteri* is unusual. We hypothesize that the native lactobacillus two-component regulatory system, signal transduction histidine kinase (LytS) and histidine kinase receptor (LytR), might be responsible for the constitutive activation of P3, as these proteins belong to the same family of both AgrA and AgrC (Supplementary Figures 2b, 3, 4).[26] In the presence of AIP-I, the phosphorylated AgrA competes with LytS for binding to P3 but is is unable to recruit the native lactobacillus transcription factors, thus inhibiting the transcription of *GusA* (Supplementary Figure 2c). This hypothesis would need to be further investigated in a follow-up study.

AIP Biosensor Kinetics. We assessed the sensitivity of the biosensors through the expression rate of the glucuronidase enzyme which is determined by the initial catalytic conversion rate inhibited in the presence of AIP-I. Using the start $\lambda_{600\ nm}$ of 0.1 and observing over a defined period of 4 h, we are able to determine the response concentration gradient of the sensors to AIP-I. Changes in the glucuronidase expression rate were detectable within the first 30 min upon introduction of AIP-I with steady catalytic conversion for the first 1.5 h. We determined that the effective reporting range of biosensor V1 occurred in the range of 10^{-8} to 10^{-7} molar concentration of AIP-I with EC50 values of 15.346 ± 1.338 nM (Figure 3a), while that for biosensor V2 was within the range of 10^{-9} molar AIP-I with EC50 values of 1.1047 ± 1.527 nM (Figure 3b). We confirmed the glucuronidase activity by investigating the glucuronidase expression level from 0.1 g of wet weight cell culture (Figure 3c and 3d). The expression level of glucuronidase decreases with the increase of AIP-I concentration. The lower expression of glucuronidase in biosensor V1 when induced with low AIP-I (<1 nM) might be attributed to the lower metabolic state in the absence of AIP-I.

For the development of the biosensor as a diagnostic tool, we determined the optimal conditions that give the best sensitivity for determination of AIP-I concentrations in water-based samples. To this end, we investigated the optimal biosensor cell density for sensing AIP-I by testing various concentrations of AIP-I against different starting λ_{600} of biosensors V1 or V2 (Figure 3e,f). Our findings support our hypothesis that changes in the biosensor sensitivity is attributed to the tightly regulated expression of *GusA* in differing AIP-I concentrations which is based on the orientation of the regulatory module and the sensing module in the constructs. Biosensor V1 was less sensitive to AIP-I inhibition and showed a gradient decrease of velocity as AIP-I increased. It functioned best at $\lambda_{600\ nm}$ of 0.05 to 0.5 giving significantly distinguishable readout for AIP-I concentration determination (Figure 3e). Biosensor V2, on the other hand, had a tighter regulation compared to V1 and could respond at higher sensitivity to low concentrations of AIP-I. Notably, the use of starting λ_{600} above 0.1 yielded a better response (Figure 3f). Henceforth, the best cell density for testing AIP-I concentrations was fixed as $\lambda_{600\ nm}$ of 0.1 as it gives good response from both sensor constructs. The AIP-I induced fluctuation of *GusA* expression rate at the different initial $\lambda_{600\ nm}$ could be due to changes of the cellular biochemistry in different bacterial growth phases. The changes of the cellular biochemistry can possibly affect how the *agr*QS respond in the sensor.[27]

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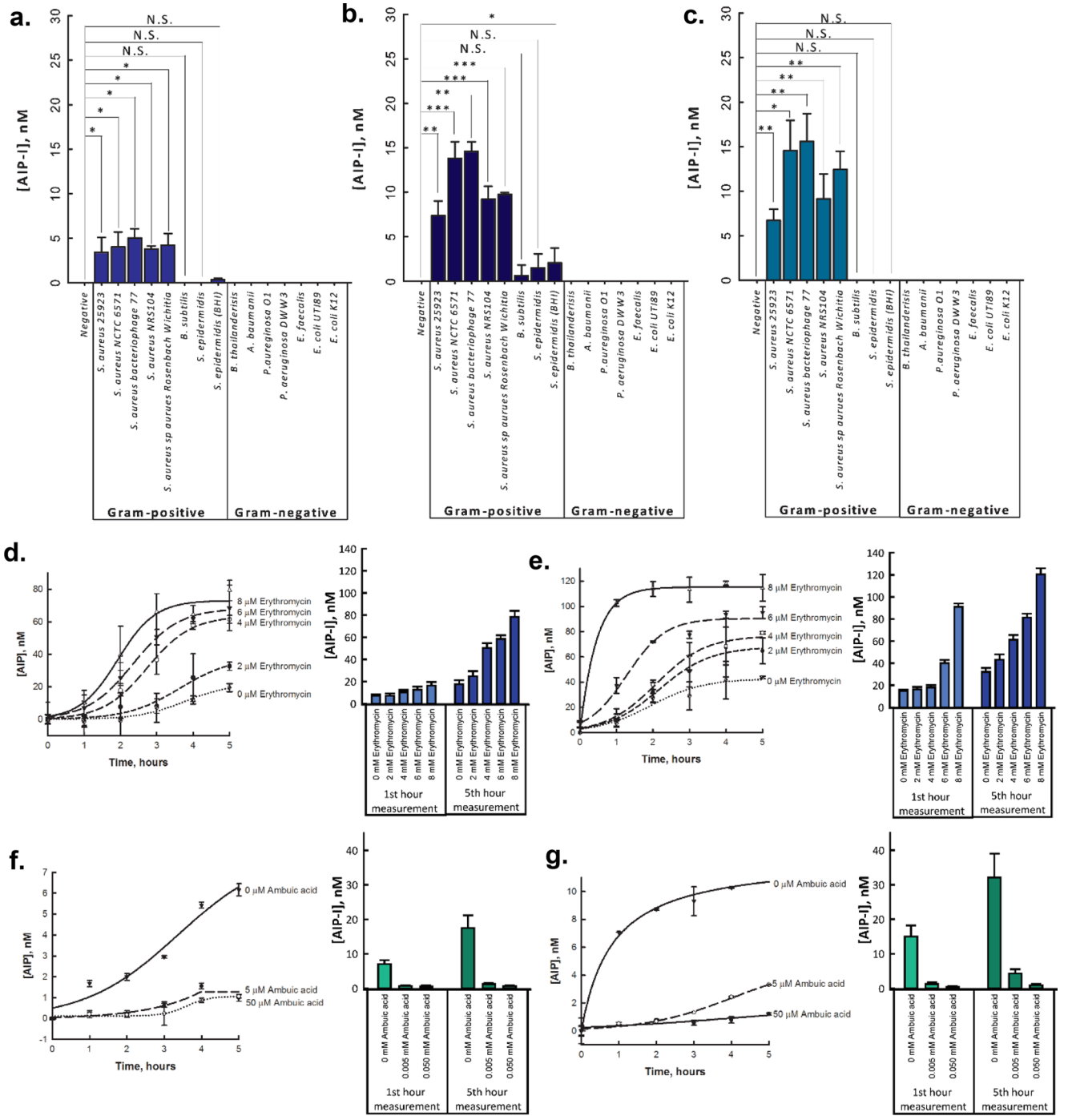

Figure 4. Response of biosensors V1 and V2 tested with supernatant of various pathogenic Gram-positive and Gram-negative microbes. Detection of AIP-I in supernatant of various Gram-positive and negative cultures grown in tryptic soy broth by biosensors (a) V1 and (b) V2. (c) Quantification of AIP-I in supernatant of various Gram-positive and negative cultures using mass-spectrometry. Cultures annotated with BHI indicate that the cells were grown in brain heart infusion media. NS represents nonsignificant,, (∗) $p \leq 0.05$, (∗∗) $p \leq 0.01$, (∗∗∗) $p \leq 0.001$, and error bars represent the standard deviation of biosensor readout using glucuronidase assay. V1 used to detect high AIP-I induced by erythromycin in (d) low AIP-I producer, *Staphylococcus aureus* 25923 and (e) high AIP-I producer, *Staphylococcus aureus* 77. Bar charts show the mass spectrometry quantified concentration of AIP-I produced by the respective *Staphylococcus aureus* during the first and fifth hour post challenge with erythromycin. V2 used to detect levels AIP-I inhibited by ambuic acid in (f) low AIP-I producer, *Staphylococcus aureus* 25923 and (g) high AIP-I producer, *Staphylococcus aureus* 77. Bar charts show the mass spectrometry quantified concentration of AIP-I produced by the respective *Staphylococcus aureus* during the first and fifth hour post challenge with ambuic acid.

Specificity and Real-Time Dynamic Reporting. The most important aspect of a biosensor is its specificity and ability to report the presence of the signal in real time. We first tested the sensor using various Gram-positive and Gram-negative pathogens culture media grown sampled after 1 h post-inoculation. Both biosensors were able to report AIP-I presence in all subspecies of *Staphylococcus aureus* and respond weakly to *Bacillus subtilis* and *Stahylococcus epidermidis*. Both sensors were

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not responsive to Gram-negative bacteria (Figure 4a,b). As the levels of AIP-I quantified from the media was relatively low, sensor V2 managed to report similar levels of AIP-I compared to levels measured using mass spectrometry (Figure 4b,c). Previous reports suggest some crosstalk between Gram-positive bacteria including those between other *Staphylococcus* and *Bacillus* genera *agr*QS.[28] This might explain the trace amount of detectable signals during the test using supernatant from *Bacillus subtilis* and *Staphylococcus epidermidis*. On the basis of the established levels of AIP-I produced by various *Staphylococcus aureus* cells propagated in a similar fashion, two *S. aureus* cell lines, a high (*S. aureus* 77) and low (*S. aureus* 25923) AIP-I producing strain, were used to test the real-time dynamic reporting. We performed the study using different stimulus to challenge the *S. aureus* cell lines to increase and decrease AIP-I levels (Figure 4d−g).

To evaluate real time AIP-I sensing, we used our sensors to determine the levels of AIP-I from *S. aureus* media supernatant challenged with erythromycin[19] or ambuic acid[29] that is known to upregulate and repress the release of autoinducer peptides, respectively. Clindamycin and erythromycin are macrolide antibiotics that induce cellular pathogenesis in *S. aureus* at subinhibitory concentrations.[19,30] This is possibly due to the upregulation of the *Erm*R gene that is expressed during the introduction of the antibiotics leading to increased levels of AIP-I.[31] On the other hand, ambuic acid inhibits cyclic peptide quorones biosynthesis in Gram-positive bacteria by inhibiting the gelatinase biosynthesis-activating pheromone. Thus, by using *S. aureus* 77 and *S. aureus* 25923 treated with an increasing concentration range of erythromycin or ambuic acid, we can determine the fluctuations of AIP-I levels in response to the added antagonist using our biosensors. Because of the complementary operational range of both the biosensors, we were able to track the changes in AIP-I levels produced by *S. aureus*. On the basis of the AIP-I concentration sensed by V1 and V2 in *S. aureus* 77 cultures treated with erythromycin and ambuic acid, V1 is able to report accurately the concentration of secreted AIP-I at the higher concentrations (Figure 4d, 4e) while V2's reading saturates upon the first hour (Supplementary Figure 5) when *S. aureus* 77 was treated with erythromycin above 2 μM. These values were compared to the mass spectrometry determination of AIP-I levels from the culture media sampled at the start (first hour) and the end point of the study (fifth hour). Similarly, V2 can detect concentrations within the nM range giving an accurate read out of *S. aureus* 77 response to ambuic acid (Figure 4f,g), while V1 is unable to give a distinguishable concentration readout (Supplementary Figure 6). The function of biosensors V1 and V2 were demonstrated in detecting AIP-I in *S. aureus* 25923 cultures treated with either erythromycin or ambuic acid.

The response of *S. aureus* 77 and *S. aureus* 25923 when treated with Erythromycin showed an increase of AIP-I. Upon closer observation, the increased levels of AIP-I post-1 h sampling of *S. aureus* 77 compared to *S. aureus* 25923 (Figure 4c,d) is due to the robustness of AIP-I biosynthesis. *S. aureus* 25923 showed an average of 1−2 h delay of AIP-I production compared to the profile of *S. aureus* 77. This suggests that while most *S. aureus* produces AIP for quorum sensing, the rate of biosynthesis varies from one to the other.[19]

On the basis of these observations, we conclude that our biosensors are able to accurately report the AIP-I concentration in a mixture for quick and high-throughput screening purposes. Current approaches for detecting and quantifying AIP-I use MALDI-TOF-TOF mass spectrometry[32] or ultrahigh performance liquid chromatography,[33] both of which require expensive equipment and consumables. Furthermore, samples have to be loaded one at a time thus requiring a long processing time for large quantities of samples. This poses a problem for detection in a clinical setting where patient samples can be numerous and the analytical results are needed promptly to make timely diagnoses.[34] Using the developed biosensors, we can rapidly determine the presence of any *Staphylococcus sp.* in human samples and the state of virulence of the microbes. Similarly, in medical research the high-throughput screening would allow researchers to identify potential candidates that target pathogenic infection. This would further propel the field of combinatorial therapy to identify suitable treatments that would synergistically work well with other treatments.[35]

CONCLUSION

We have produced a pair of whole cell biosensors to detect AIP-I at a concentration range of 10^{-9} to 10^{-7} molar. With sensitivity to concentrations as low as 0.5 nM AIP-I, these biosensors would be useful for qualitative and quantitative detection of *Staphylococcus* in a medical setting allowing for swift decision making in regards to the treatments to be administered. Further, the biosensor provides a cost-effective method for the simultaneous screening of a high number of patient samples. On top of this, our biosensor system could be performed in a high-throughput manner potentially making drug discovery and investigations on alternative therapy more efficient. The sensor could be further developed as a suitable chassis for engineering a probiotic to combat pathogenic *Staphylococcus* infection by coupling the sensing function to a suitable response output.

MATERIALS AND METHODS

Bacterial Strains, Plasmids, and Growth Conditions. *Escherichia coli* Top 10 (Invitrogen) was used for cloning, and *Lactobacillus reuteri* DSM 20016 (DSMZ) was used for the expression of sensors V1 and V2. A shuttle vector, pTRKH3-slpGFP was utilized to enable cloning within *E. coli* and propagation in *Lactobacillus reuteri*. *E. coli* were grown in Lysogeny broth (Tryptone 10 g L^{-1}, yeast extract 5 g L^{-1}, sodium chloride 10 g L^{-1} purchased as premixed media from BD) at 37 °C, 200 rpm. *L. reuteri* were grown in MRS medium (proteose peptone no. 3 10 g L^{-1}, beef extract 10 g L^{-1}, yeast extract 5 g L^{-1}, dextrose 20 g L^{-1}, polysorbate 80 1 g L^{-1}, ammonium citrate 2 g L^{-1}, sodium acetate 5 g L^{-1}, magnesium sulfate 0.1 g L^{-1}, manganese sulfate 0.05 g L^{-1}, dipotassium phosphate 2 g L^{-1}, purchased as premixed media from BD) at 37 °C in stationary conditions. BD agar was used to a final concentration of 1.5% to make plates. Where required erythromycin was added at a concentration of 250 μg mL^{-1} for *E. coli* and 10 μg mL^{-1} for *L. reuteri*.

AIP Stock Solutions, Bacterial Cells, and Antagonist Used. Purified synthetic AIP-I was synthesized by Cambridge Peptides and dissolved in distilled water to make a stock solution of 500 μM. Various strains of *S. aureus* and other microbes (Supplementary Table 1) was grown for 3 h unless stated otherwise in LB broth at 37 °C, 200 rpm. Cultures were centrifuged, and the supernatant was filter sterilized. For the AIP-I producing assay, ambuic acid and erythromycin (Sigma) were used at different concentrations.

Plasmid Constructions. Plasmids were constructed *via* Gibson assembly (Gibson assembly master mix, NEB no. E2611S). Primers were designed to contain 25 bp overhangs

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for homologues recombination and were purchased from IDT Integrated DNA Technologes. Phusion polymerase (Thermo-Fisher Scientific, no. F534S) was used for all amplifications. V1 was constructed by amplifying the agrCA genes from *S. aureus* RN 4220. The promoter p3 was amplified from the *S. aureus* RN 4220 genome. The reporter gene GusA was amplified from pSIP409 including the RBS site. All parts were transformed into *E. coli* Top 10 and sequence verified. V2 was constructed by flipping slp-AgrCA from V1 *via* PCR amplification and Gibson assembly.

Transformation of *L. reuteri*. The synthetic constructs were transformed into *L. reuteri via* electroporation as described previously by Berthier *et al.* The following modifications have been made to increase efficiency: Colonies were grown at 37 °C in MRS media overnight under stationary conditions. On the next day, cultures were inoculated (1:50) into MRS media containing 2.5% glycine and 0.25 M sucrose to weaken cell wall and add osmotic pressure. Cultures were incubated stationary at 37 °C until OD 0.3−0.5 followed by the procedure described by Berthier. For electroporation, 1 μg of plasmid DNA in a maximum of 5 μL of ddH_2O was added to 50 μL cell suspension. This mixture was transferred in a 0.2 cm cuvette and electroporated with 1.8 kV pulse and 800 Ohm resistance (Bio-Rad Gene Pulser) followed by recovery and outgrowth as described in the original protocol from Berthier.

GusA Assay. GusA assay was conducted as previously described with minor modifications. Colonies were grown in MRS media containing appropriate antibiotics overnight at 37 °C without shaking and inoculated in fresh media at 1:1000 the next day. After samples reached an OD of 0.5−0.9, 500 μL aliquots were taken and centrifuged at 5000 rpm for 5 min. The supernatant was discarded, and pellets were resuspended in 50 μL of sodium phosphate buffer (pH = 7). Following, 450 μL of GUS buffer (50 mmol L^{-1} NaH_2PO_4, pH 7.0, 10 mmol L^{-1} β-mercaptoethanol, 1 mmol L^{-1} ethylenediaminetetraacetic acid, and 0.1% Triton X-100) was then added to each sample. This was mixed with 12.5 μL of 0.1% sodium dodecyl sulfate and 25 μL of chloroform. After 10 min in a 37 °C water bath, *p*-nitrophenyl-β-D-glucuronide substrate (4 mg mL^{-1} in 50 mmol L^{-1} NaH_2PO_4 (pH = 7)) was added. After mixing, this was incubated further at 37 °C. The reaction was stopped by addition of 250 μL of 1 mol L^{-1} Na_2CO_3. The $\lambda_{405\ nm}$ value of the supernatant was measured using a BioTek Synergy H1 microplate reader. Activity was calculated as described by Miller (1972) and expressed as Miller Units equivalents (MU). Mean results of triplicate samples are reported.

Kinetic Assay. Cell cultures of sensors V1 and V2 were prepared to λ_{600} of 0.1 prior to incubation with various concentrations of purified AIP-I (0 to 10 000 nM). The initial velocity of β-glucuronidase activity was determined through time course sampling for a period of 3 h by calculation of the slope at the start of the reaction. The initial velocity is plotted against the AIP-I concentration to determine the half maximal effective concentration of sensors V1 and V2. To validate the expression levels of β-glucuronidase, 100 mg of wet weight cell mass of each treatment group were lysed using lysozyme coupled with sonication-mediated cell rupture for the determination of β-glucuronidase expression levels. Western blot was performed and stained with mouse anti-β-glucuronidase antibody (AB188492) and costained with goat antimouse antibody conjugated with horseradish peroxidase (ab97023). Western blot was developed with chemiluminescent HRP substrate (ab5801). To investigate the changes of AIP-I induced expression level when the biosensor culture were induced at different $\lambda_{600\ nm}$, the assay was conducted using λ_{600} 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, and 5.0 tested on various concentration of purified AIP-I (0 to 10 000 nM). A heat map measuring the initial velocity of the β-glucuronidase was plotted to determine the optimal cellular density for detection of AIP-I.

Measurement of AIP-I Levels in Various Microbial Cultures. Cell cultures of various microbes (shown in Supplementary Table 1) were grown at 37 °C, 200 rpm for 3 h before harvesting, where the supernatant was used for measuring the AIP-I concentration following the protocols above. Concentrations were performed by comparing the measured $\lambda_{405\ nm}$ in the linear range of the established response curves of the biosensor to the different AIP-I.

Perturbation of AIP-I levels in *Staphylococcus aureus* by Subinhibitory Concentrations of Erythromycin and Ambuic Acid. *Staphylococcus aureus* cultures were initially inoculated at $\lambda_{600\ nm}$ = 0.1 and increasing concentration of erythromycin and ambuic acid was added. Changes of AIP-I levels were measured hourly over the period of 5 h.

Mass Spectrometry Quantification of AIP-I. Supernatant from bacterial cultures were concentrated by freeze-drying and reconstituted in ultrapure water to make 10× concentration. Samples were filtered using 0.2 μm cartridge filter (Sartorius) prior to injection. Samples were analyzed using liquid chromatography tandem mass spectrometry 6550 iFunnel QTOF LC/MS (Agilent) equipped with Eclipse Plus C18 RRHD 1.8 μm (2.1 mm × 50 mm) column (Agilent), using the Dual AJS ESI ion source, scanning for positive ion polarity. Briefly, 10 μL of sample was injected into the column and separated using an isocratic run of 20% acetonitrile with 0.1% formic acid with a gradient increase to 60% acetonitrile with 0.1% formic acid in a duration of 5 min, followed by a gradient increase to 100% acetonitrile with 0.1% formic acid in a duration of 30 s. The ionization profile was detected for mass m/z 961.3794 ± 0.0048 [M+H+]. The ionization profile correlates the standard AIP-I profile. AIP-I standards at varying concentrations were used as a reference to quantify the AIP-I isolated from the concentrated bacterial cultures. Student's t test was used in the statistical analysis.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssynbio.8b00063.

Supporting methods, tables, and figures (PDF)

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supervised the project. All authors discussed the results and commented on the manuscript.

Notes

The authors declare no competing financial interest.

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