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Development of *Corynebacterium glutamicum* as Staphylococcal-Targeting Chassis via the Construction of Autoinducing Peptide (AIP)-Responsive Expression System

(Pembangunan *Corynebacterium glutamicum* sebagai Casis Penyasaran Staphylococcos melalui Pembinaan Sistem Pengekspresian Responsif terhadap Peptida Autoaruhan (AIP))

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ABSTRACT

Despite increasing reports of antimicrobial activities of commensal and non-pathogenic bacteria such as *Corynebacterium* spp., previous studies on bioengineered therapeutics traditionally employed probiotics and food-grade bacteria which limits further advancements into microbial therapeutics research. In this study, *Corynebacterium glutamicum*, a generally recognised as safe (GRAS) and model bacterium was employed as a new chassis for the development of bioengineered corynebacterial chassis tailored towards *Staphylococcus* sp. via autoinducer peptide (AIP)-based quorum sensing (QS) interactions. To develop *C. glutamicum* as a staphylococcal-targeting chassis, the bacteria were transformed with the pResponse plasmid harboring AIP-responding accessory regulatory proteins *agrAC* and red fluorescent protein (RFP) genes under the control of the P*aceA* and P3 promoter, respectively, which was expected to stimulate the production of fluorescence signals in the presence of AIPs. Fluorescence activity of the *C. glutamicum* pResponse strain was compared to control *C. glutamicum* pRFP strain containing only the P3-RFP gene without the *agrAC* gene cassette. Using AIP-I as the input biomolecule, *C. glutamicum* pResponse strain fluoresced under different concentrations of AIP-I whereas no fluorescence was observed in the control *C. glutamicum* pRFP strain. When tested with *S. aureus* culture supernatant, the pResponse strain exhibited increasing fluorescence over the incubation period with the highest fluorescence signal of 183 relative fluorescence units (R.F.U) was observed at the 48 h point thereby demonstrating a functional QS-responsive protein expression system in bioengineered *C. glutamicum*. These findings demonstrated the feasibility and promising potential of developing bioengineered *C. glutamicum* as a staphylococcal-responsive and -targeting chassis.

Keywords: AIP signaling; bioengineered chassis; biological engineering*; Corynebacterium glutamicum*; *Staphylococcus aureus*; synthetic biology

ABSTRAK

Walaupun terdapat pertambahan pelaporan berkenaan aktiviti antimikrob oleh bakteria komensal dan bukan patogen seperti *Corynebacterium* spp., kajian terdahulu dalam penghasilan terapeutik terjurutera biologi secara tradisinya memfokuskan kepada penggunaan probiotik dan bakteria gred makanan yang mengekang perkembangan kemajuan dalam kajian terapeutik mikrob. Dalam kajian ini, *Corynebacterium glutamicum* iaitu sejenis bakteria model dan dianggap sebagai bakteria selamat (GRAS), telah digunakan untuk pembangunan casis baharu Corynebacteria yang bertindak balas dan mengaruh khusus kepada bakteria spesies *Staphylococcus* melalui tindak balas isyarat penderiaan kuorum (QS) berasaskan peptida autoaruhan (AIP). Bagi tujuan ini, bakteria *C. glutamicum* telah ditransformasi dengan

plasmid pResponse yang mengandungi gen aksesori kawal atur (agr), *agrAC* dan protein berpendarfluor merah (RFP) yang masing-masing di bawah kawalan promoter P*aceA* dan P3 yang dijangkakan akan merangsang penghasilan isyarat pendarflour dengan kehadiran AIP. Aktiviti penghasilan pendarflour oleh strain pResponse *C. glutamicum* dibandingkan dengan aktiviti strain kawalan pRFP *C. glutamicum* yang hanya mengandungi gen P3-RFP tanpa kaset gen *agrAC*. Melalui asai menggunakan sebatian AIP-I, strain pResponse *C. glutamicum* menghasilkan isyarat pendafluor namun tidak bagi strain kawalan pRFP apabila diuji dengan kepekatan berbeza AIP-I. Apabila diuji dengan sampel supernatan daripada *S. aureus*, strain pResponse mengeluarkan isyarat pendarfluor yang berkadaran dan selari dengan tempoh eraman. Bacaan isyarat pendarfluor tertinggi oleh pResponse adalah 183 unit pendarfluor relatif (R.F.U) pada jam ke-48 yang menunjukkan bahawa sistem penghasilan protein berasaskan tindak balas QS AIP-I ini telah berjaya diimplementasikan dalam *C. glutamicum*. Hasil penemuan daripada kajian ini telah menunjukkan kebolehlaksanaan dan potensi besar penggunaan *C. glutamicum* terjurutera biologi sebagai casis pengesan dan pensasar bakteria jenis *Staphylococcus*.

Kata kunci: Biologi sintesis; casis terjurutera biologi; *Corynebacterium glutamicum*; isyarat AIP; kejuruteraan biologi; *Staphylococcus aureus*

Introduction

The emergence of new infectious diseases and drug-resistant pathogens has led to greater need for new drug design and production pipelines. Over the past decade, the use of commensal and probiotic microbes has gained increasing attention in synthetic biological applications and live biotherapeutic products (LBPs) development, particularly as next-generation biotechnological advancement in diagnostics and therapeutic interventions for human diseases (Charbonneau et al. 2020; Ozdemir et al. 2018). LBPs principally involve the employment of live microbes that are either native or genetically altered for the prevention and treatment of human diseases (Ducarmon, Kuijper & Olle 2021; Ozdemir et al. 2018).

Advances in biological engineering and synthetic biology have accelerated the development of bioengineered commensal, probiotic and attenuated bacteria for disease prognosis, monitoring or curing through specific biomolecules interaction and targeted killing of disease-causing pathogens (Charbonneau et al. 2020; Rottinghaus, Amrofell & Moon 2020). Through bioengineering strategies, various microbial hosts especially probiotic strains have been engineered to express sensory and/or therapeutic proteins as therapeutic intervention for human diseases including inflammatory disease, diabetes, cancers and bacterial infections (Inda et al. 2019; Rottinghaus, Amrofell & Moon 2020). Generally, probiotic bacterial strains are used to produce pathogen-targeting proteins via the introduction of genetic tools including native and heterologous regulatory elements such as constitutive promoters and metabolite-responsive transcription factors (Rottinghaus, Amrofell & Moon 2020; Zuo, Chen & Marcotte 2020). In an effort to provide precise microbiome interaction and targeting, a seek-and-kill approach was iteratively employed by allowing specific regulation and control of pathogen killing or in some cases, the host, through environmental or quorum sensing (QS) stimuli that act as inducers to initiate targeted pathogen elimination (Rottinghaus, Amrofell & Moon 2020; Tan et al. 2020). Generally, engineered therapeutic microbes are designed to target the disease or pathogen through metabolite-responsive expression systems in tandem with the production of outputs in the form of reporter and therapeutic proteins.

Several probiotic bacterial hosts from *Lactobacillus*, *Bifidobacterium* and *Lactococcus* groups as well as *Escherichia coli* Nissle 1917 have been bioengineered to target pathogenic bacteria such as *Vibrio cholerae*, *Pseudomonas aeruginosa*, *Salmonella* sp. and enterotoxigenic *E. coli* (Inda et al. 2019; Rottinghaus, Amrofell & Moon 2020; Tan et al. 2020). Considering the diverse microbiome communities and evolving environmental cues in stimulating infectious disease, there is an urgent need for the expansion of bacterial hosts especially using food-grade bacteria with readily-available genetic toolsets, well-established metabolic understanding and free from intrinsic virulence factors (Charbonneau et al. 2020). Compared to the current probiotic strains, new bacterial chassis could be obtained from generally regarded as safe (GRAS) model microbes and strains associated with the human microbiome which could be particularly useful for non-oral applications and topical treatment of skin diseases.

Corynebacterium glutamicum is a GRAS and model bacteria for molecular studies and biotechnological applications especially in the production of high-value active ingredients for food, feed and pharmaceuticals (Sanchez et al. 2018; Wolf et al. 2021). Expansive

and increasingly available genetic parts and genome editing tools in *C. glutamicum* have rendered strain development using this bacterial chassis highly desirable and advantageous (Wang et al. 2021). To date, there is no report that can be found on the use of *C. glutamicum* as a pathogen-targeting bioengineered chassis despite the recent discovery of pathogen-inhibiting activities of *Corynebacterium* spp. that act as non-pathogenic commensal bacteria in the human skin microbiome (Bomar et al. 2016; Byrd et al. 2018; Ramsey et al. 2016). *C. accolens* was shown to interact and produce free fatty acids as part of the corynebacterial anti-pneumococcal activity against pathogenic *Streptococcus pneumoniae* (Bomar et al. 2016; Menberu et al. 2021). This has contributed to the growing research on the use of skin commensal bacteria such as engineered *Staphylococcus epidermidis* that were tested for protective and biotherapeutic potential against pathogenic *S. aureus*, the main causative agent of inflammatory skin diseases including atopic dermatitis (Dodds et al. 2020; Tham et al. 2020).

More importantly, previous work on staphylococcal-targeting constructs was carried out using non-corynebacterial hosts hence limiting the potential benefits of harnessing skin commensal bacteria as potential LBPs for therapeutic intervention of staphylococcal infection. With the aim of developing *C. glutamicum* as a new bioengineered chassis for staphylococcal pathogens, this study was specifically designed to generate a staphylococcal-specific QS-directed expression system in bioengineered *C. glutamicum*. In this study, co-culture assays and biological engineering of *C. glutamicum* were carried out to determine anti-staphylococcal activity and develop a staphylococcal-responsive expression system through the production of recombinant red fluorescent protein (RFP) as QS-transducing signal output. Using autoinducing peptide (AIP)-based QS interactions, *C. glutamicum* was engineered to be highly responsive to AIP-I QS peptide from *S. aureus* for directing the heterologous protein expression in the form of the fluorescence-emitting RFP protein. Through assays with AIP-I and *S. aureus* supernatant, this study demonstrated proof-of-concept for the development of skin commensal corynebacteria as new bioengineered chassis especially for relevant skin disease studies and LBP-based intervention. To the best of our knowledge, this study is the first to report the development of bioengineered *C. glutamicum* as a staphyloccal responsive and targeting chassis using biological engineering approaches.

Materials and Methods

Strains and culture conditions

C. glutamicum (DSM 102071) was employed as bacterial chassis for cultivation, the co-culture and bioassay experiments (Table 1). *E. coli* DH5α was used for molecular cloning and plasmid maintenance while *S. aureus* RN4220 was used in co-culture and bioassay experiments. Luria Bertani (LB), Mannitol Salt Broth (MSB) and Tryptic Soy Broth (TSB) and 1% glucose media with kanamycin (50 µg/mL) was used for appropriate bacterial growth and plasmid propagation experiments. Cultivation for *E. coli* and *S. aureus* were mainly carried out at 37 °C (30 °C was used for mono- and co-culture assays of *S. aureus*) while *C. glutamicum* was cultivated at 30 °C under aerobic conditions.

Co-culture assays of *C. glutamicum* and *S. aureus*

Co-culture and anti-staphylococcal activity assay were done by measuring colony forming units (CFU) of *S. aureus* grown on mannitol salt agar (MSA) plates which only allows the growth of *S. aureus* but not *C. glutamicum*. Briefly, seed cultures of *C. glutamicum* and *S. aureus* were grown overnight (20 h) in TSB broth at 30 °C and used to inoculate fresh TSB broth (OD_{600}: 0.1) either individually (*C. glutamicum*; negative control and *S. aureus*; positive control) or co-culture samples of *C. glutamicum*-*S. aureus* that were incubated in 15 mL tubes at 30 °C, 200 rpm for 20 h. Following cultivation, serial dilution was carried out and the corresponding samples were plated onto MSA plates for subsequent incubation at 30 °C. Cell concentrations (CFU/mL) of individual and co-culture plates was determined after 3 days of incubation. Lysis spot assay of *C. glutamicum*-*S. aureus* coculture was carried out using methods previously described (Kim, Yoon & Choi 2015) of which different volumes of wild type *C. glutamicum* samples were spotted on *S. aureus*-containing LB plates.

Construction of plasmids

The staphylococcal QS-responsive expression system was constructed in *C. glutamicum* via plasmid expression using the pBL1 replicon-based pJYS1Peftu plasmid (Jiang et al. 2017). A synthetic gene fragment containing a modified P3 promoter (S. *aureus*), codon-optimised RFP (*Discosoma* sp.) and rrnB T1T2 terminator (*E. coli*) was chemically-synthesised as a gBlock gene fragment (Integrated DNA Technologies, USA). The AIP-responsive

construct was generated by isothermal assembly of the P*aceA* promoter (*C. glutamicum*), *agrAC* genes (*S. aureus*), P3-RFP and vector backbone fragments to yield the pResponse plasmid. The control plasmid pRFP was constructed by ligating PCR-amplified DNA fragments of the plasmid backbone and the P3-RFP gene cassette via isothermal assembly using *E. coli* DH5α as cloning host (Ramzi et al. 2018). The plasmid constructs were confirmed via Sanger sequencing and transformed into *C. glutamicum* competent cells using electroporation as previously described (Ramzi et al. 2015; Van der Rest et al. 1999;). Positive transformants of pResponse and pRFP *C. glutamicum* strains were confirmed via Colony PCR. All bacterial strains, plasmids and primers used in this study are listed in Table 1.

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

Strain or plasmid	Genotype or construct	Reference or source
Bacterial strains		
E. coli DH5α	F^{-}, *deoR*, *endA1*, *gyrA96*, *hsdR17*(rk^{-}mk^{+}), *recA1*, *relA1*,*supE44*, *thi-1*, Δ(*lacZYA- argF*)*U169*, (Phi80*lacZ*delM15)	Invitrogen[a]
C. glutamicum MB001 (DE3)	Prophage-free derivative of *C. glutamicum* ATCC 13032 with chromosomal expression of T7 RNA polymerase gene; DSM 102071	DSMZ[b]
S. aureus RN4220	Wild type	ATCC[c]
Plasmids		
pJYS1Peftu plasmid	*E. coli-C. glutamicum* shuttle vector, Km^{R}, pBL1 replicon	(Jiang et al. 2017)[d]
pResponse	pJYS1Peftu whereby FnCpf1 and recT replaced with DNA fragments containing P*aceA* promoter (*C. glutamicum*), *agrAC* (*S. aureus*), P3 promoter (S. *aureus*) and codon-optimized RFP (*Discosoma* sp.)	This study
pRFP	pResponse plasmid without P*aceA* promoter (*C. glutamicum*) and *agrAC* (*S. aureus*)	This study

Amp, ampicillin; Km, kanamycin; R, resistance. [a]Invitrogen Corporation, Carlsbad, CA, USA. [b]Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, GERMANY. [c]American Type and Culture Collection, Manassas, USA. [d]pJYS1Peftu was a gift from Sheng Yang (Addgene plasmid # 85546)

FLUORESCENCE ACTIVITY ASSAY

To demonstrate staphylococcal QS-responsive ability of bioengineered *C. glutamicum*, *agrAC*-harboring pResponse and control pRFP *C. glutamicum* strains were tested with pure AIP-I (YSTCDFIM; ProteoGenix, France) and *S. aureus* supernatant samples. For the AIP-I assay, overnight culture of pResponse and pRFP *C. glutamicum* cells (OD_{600nm} = 0.4 to 0.5 approximately) were mixed with varying concentrations of AIP-I specifically 0.02, 0.04, 0.1, 0.2 and 0.4 mM AIP-I in a total reaction mixture of 200 µL. The reaction mixtures were incubated at 30 °C with shaking (200 rpm) and assayed for fluorescence

signal production after 48 h of incubation. To determine staphylococcal-responsive bioactivity using AIP-containing *S. aureus* supernatant, *S. aureus* cells were cultured in MSB broth until OD_{600nm} reached 2 to 2.5 (stationary phase) following which the cells were filter-sterilised with a 0.22 µM filter. The resultant supernatant (100 µL) was mixed with an overnight culture of *C. glutamicum* (OD_{600nm} = 0.8 to 0.9 approximately) and the tubes were incubated at 30 °C with shaking for different incubation periods over 48 h. Fluorescence of all test samples was read using QFX fluorometer (DeNovix, USA) after removing the background fluorescence by subtracting the reading of the blank.

Results and Discussion

Development of *C. glutamicum* as Staphylococcal Targeting Bioengineered Chassis

A key aspect of developing a bioengineered bacterial chassis as an LBP is the capacity of the selected host to inhibit the growth of the targeted pathogen through endogenous or heterologous biomolecule production. *C. glutamicum* is a closely related strain of common skin-associated corynebacteria such as *C. accolens* and *C. pseudodiphtheriticum* that have been shown to inhibit the growth of *S. aureus in vitro* (Kiryukhina et al. 2013; Menberu et al. 2021). The innate ability of corynebacteria in displaying anti-staphylococcal activity represents an important trait in the potential development of genetically tractable *C. glutamicum* for therapeutic intervention of skin pathogens especially *S. aureus*. To confirm anti-staphylococcal activity of *C. glutamicum*, co-culture assays were performed and the growth of individual and co-culture samples of *C. glutamicum* and *S. aureus* was evaluated. Using MSA as selection media, *S. aureus* grown together with *C. glutamicum* was expected to have lower cell concentrations (CFU/mL) compared to individually grown *S. aureus* plates.

As shown in Figure 1(a), reduction of *S. aureus* cell concentration was noted when grown together with *C. glutamicum* (1.4×10^9 CFU/mL) compared to control *S. aureus* samples (3.6×10^9 CFU/mL) indicating the growth-inhibiting activity of *C. glutamicum* towards the pathogenic *S. aureus* strain. The growth inhibition activity occurred in the co-culture broth of which both bacteria were inoculated at the early log phase (OD_{600nm} = 0.1). This growth-associated competition contributed to the marked reduction of staphy colonies on the MSA plates. No growth of *C. glutamicum* was observed when plated on the selective MSA plates. When tested with lysis assay, there was no significant clear zones observed on *S. aureus*-containing LB plates when spotted with different volumes of wild type *C. glutamicum* (Data not shown). These findings indicated that the growth inhibiting activity of *C. glutamicum* towards *S. aureus* may be attributed to the interspecies competition between the two bacterial strains and not through endopeptidase mediated cell lysis as previously reported in the co-cultures of *Pseudomonas aeruginosa* and *S. aureus* (Kim, Yoon & Choi 2015; Mashburn et al. 2005). The antagonistic activity between *S. aureus* and other corynebacterial species specifically *C. striatum* was associated with competition and the attenuation of virulence factors under the control of the *S. aureus* accessory gene regulator (*agr*) operon where the *agr*-regulated QS system was negatively regulated in response to corynebacteria-produced products (Ramsey et al. 2016). The antagonistic relationship has been shown to occur through contact and non-contact interactions between corynebacteria and staphylococci (Hardy et al. 2020; Kiryukhina et al. 2013; Menberu et al. 2021; Ramsey et al. 2016) in which the anti-staphylococcal activity of *C. pseudodiphtheriticum* was attributed to commensal competition via the use of virulence components of *S. aureus* (Hardy et al. 2019).

Based on the innate capacity of *C. glutamicum* to reduce the growth of skin commensal *S. aureus*, further modulation of *C. glutamicum* is required for its use as a topical probiotic and live vehicle in specific therapeutic applications. For this purpose, a specially devised bioengineering strategy was implemented to develop *C. glutamicum* as a programmable and metabolite-responsive bacterial chassis specific towards *S. aureus*. Taking advantage of AIP-based inter- and intra-species QS interactions in staphylococcal bacteria, a heterologous QS-responsive system tailored to the availability of AIP-I peptide would be ideal in reprogramming *C. glutamicum* as a staphylococcal targeting bacterial chassis. To achieve this, recombinant pResponse plasmid was constructed as a staphylococcal responsive expression system incorporating *S. aureus*-derived agrAC regulatory proteins that activate the transcriptional response of the P3 promoter (Figure 1(b)). To demonstrate AIP-I-directed protein production in *C. glutamicum*, the RFP gene encoding the recombinant red fluorescent protein (*Discosoma* sp.) was cloned and designed to be under the control of the P3 promoter (*S. aureus*) where the transcriptional activity of the P3 promoter was stimulated via the agrAC QS interactions. The presence of AIP-I, the main QS metabolite from *Staphylococcus* bacteria, will serve as the main input or inducer that will

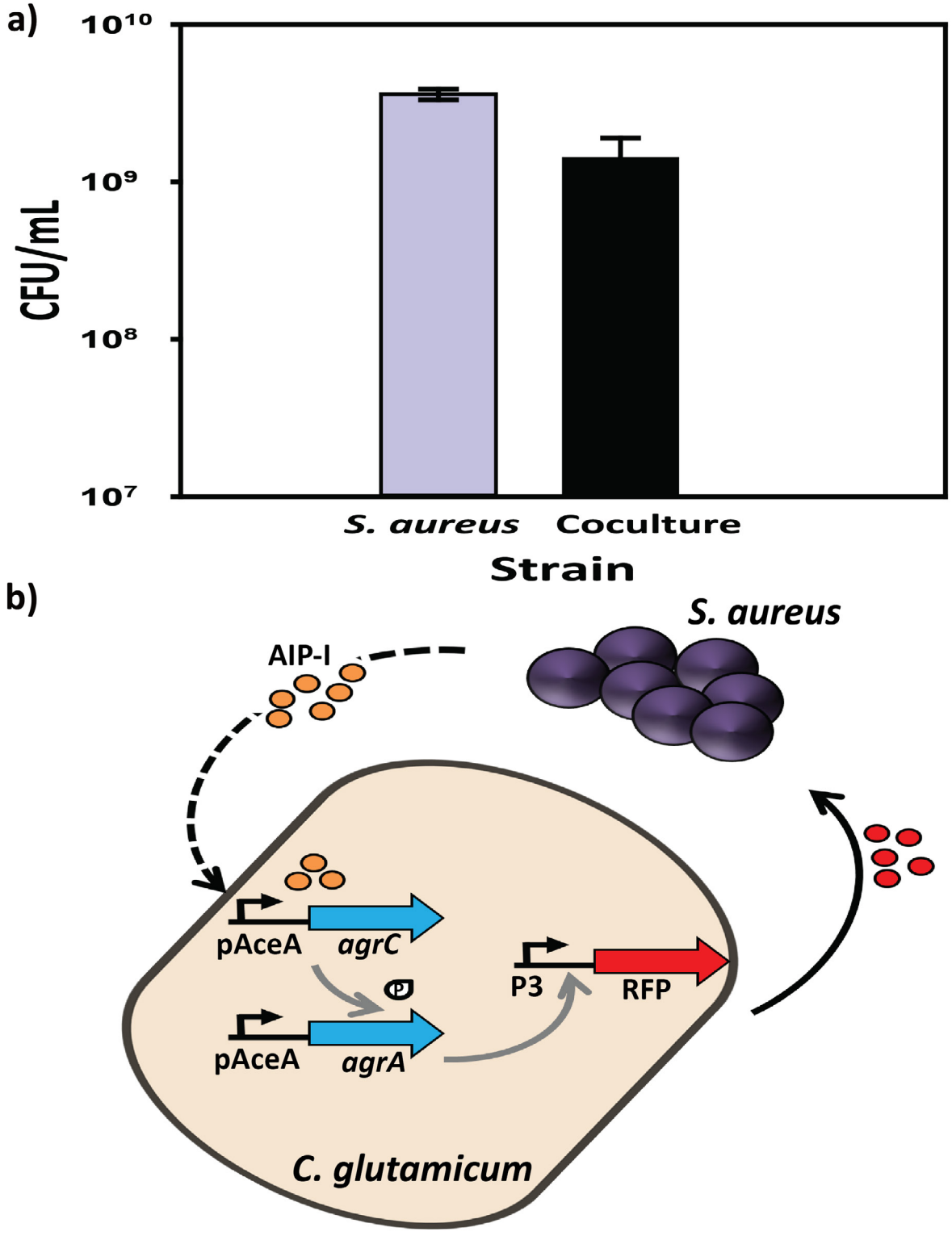

FIGURE 1. a. Growth-inhibiting activity of wild type *C. glutamicum* cells towards *S. aureus*. Serially diluted cultures of individual *S. aureus* and co-culture (*S. aureus* vs *C. glutamicum*) were plated and cell concentrations (CFU/mL) were recorded for selective growth on MSA plates. Values and error bars represent the mean and standard deviation of at least three biological replicates. b. Schematic overview of the microbial engineering strategy employed in this study. *S. aureus*-inhibiting *C. glutamicum* bacteria were selected for the construction and implementation of AIP-I-directed expression system via the introduction of the pResponse plasmid harboring accessory gene regulator, *agrAC*, and RFP genes under the control of P*aceA* and P3 promoters, respectively

transduce membrane protein agrC which phosphorylates agrA, which in turn stimulates RFP gene expression via the activation of P3 promoter. The pRFP plasmid which harbored only the P3-RFP gene cassette without the agrAC sensory proteins was used as the control plasmid in the fluorescence assays.

FLUORESCENCE PRODUCTION BY AIP-I-RESPONSIVE BIOENGINEERED *C. glutamicum*

In this work, the bioengineered *C. glutamicum* pResponse strain was designed to produce RFP protein in response to the availability of *S. aureus* AIP-I through the cascade reactions mediated by *agrC*, *agrA,* and P3-controlled RFP expression, respectively. As shown in Figure 2(a), the fluorescence signal was produced only by *C. glutamicum* pResponse strains whereas no fluorescence was observed in the control pRFP strain when tested with different concentrations of AIP-I. The highest fluorescence signal of 51 relative fluorescence units (R.F.U) was recorded in pResponse strains treated with 0.04 mM of AIP-I. These observations confirmed that the pResponse construct was an AIP-I-responsive

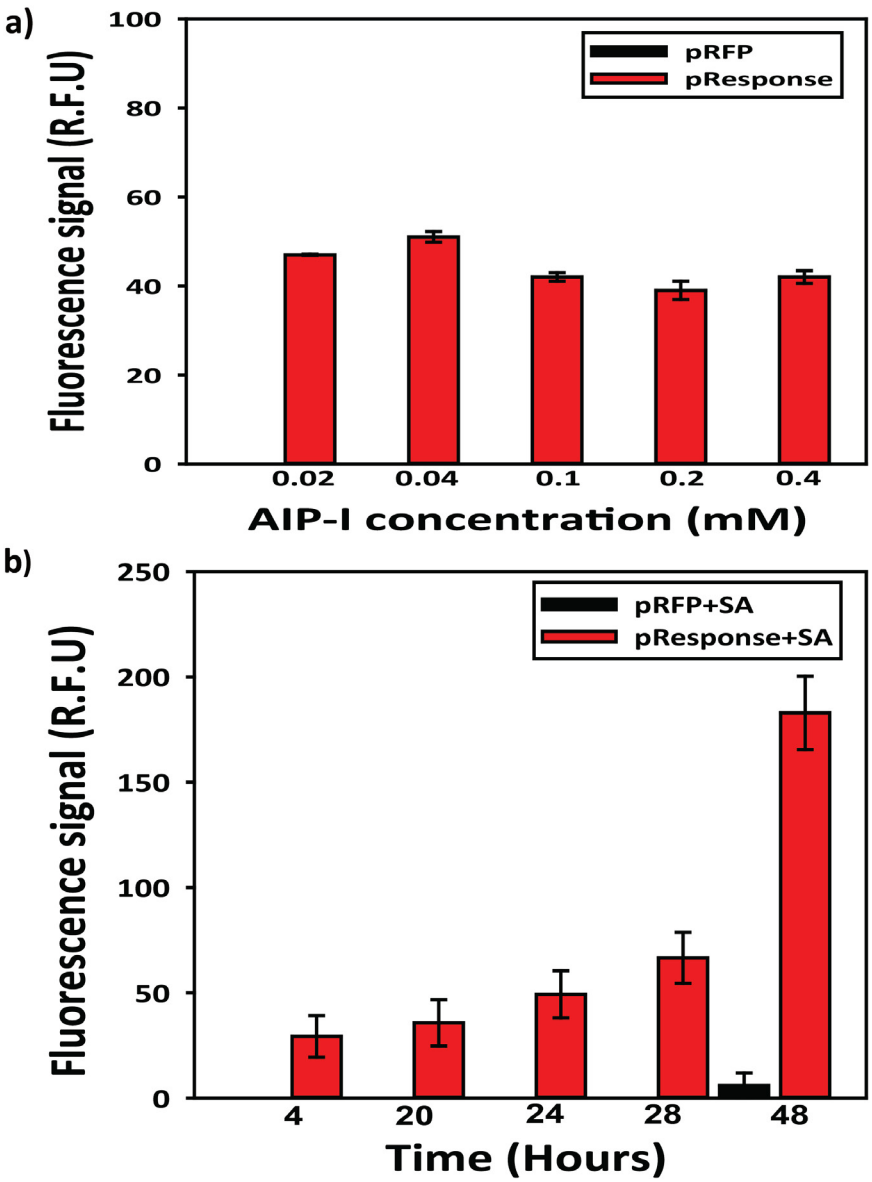

FIGURE 2. a. Fluorescence signals produced by *C. glutamicum* pResponse strains when tested with different concentrations of synthetic AIP-1 peptide. Bar graph represents the mean fluorescence signal (Relative fluorescence units; R. F. U) that was normalised relative to basal levels of expression at 0 µL AIP-I, with error bars indicating one standard deviation over three biological replicates. b. Time-dependent fluorescence of bioengineered *C. glutamicum* strains tested with and without *S. aureus* supernatant (SA). Relative fluorescence signal was only detected for *C. glutamicum* strains supplied with SA and not observed in bacterial samples without exogenous SA. Bar graph represents the mean fluorescence signal (R. F. U) that was normalised relative to basal levels of expression at 0 hour, with error bars indicating one standard deviation over three biological replicates

expression system through the signal-transducing activities of agrAC receiver proteins and subsequent activation of P3 promoter that controlled the expression of signal-emitting RFP in engineered *C. glutamicum*. The AIP-I-transducing activities of agrAC proteins in heterologous bacterial hosts have been previously shown to be responsive and important in detecting the availability of *S. aureus*-targeted AIP biomolecules (Lubkowicz et al. 2018; Marchand & Collins 2013). AIP signal transduction occurred through the presence of AIP-I peptide which activated agrC which led to the phosphorylation of agrA and the subsequent binding of the P3 promoter that controlled the expression of *agr*-associated virulence factors in *S. aureus* (Butrico & Cassat 2020; Le & Otto 2015).

To further characterise the AIP-1-mediated expression system in bioengineered *C. glutamicum*, the pResponse and pRFP strains were tested with *S. aureus* metabolites present in the *S. aureus* culture supernatant. When treated with *S. aureus*-derived supernatant, the fluorescence of pResponse strains increased over time from 4 to 48 h (Figure 2(b)). The highest RFP production was attained at the 48 h point of which about 183 R.F.U was observed in pResponse samples while fluorescence from pRFP strains was only observed at the 48 h point (6 R.F.U). No fluorescence signal was detected for both pRFP and pResponse strains when the bioengineered corynebacteria were grown without *S. aureus* supernatant thus confirming the expression of agrAC that was driven by *S. aureus* QS metabolites. The use of P*aceA*-controlled agrAC genetic parts can therefore be optimised and utilised for the expression of biotherapeutic proteins and QS biomolecules in bioengineered *C. glutamicum*. The *S. aureus*-originated agrAC parts have been employed in previous constructs in engineered *B. megaterium* (Marchand & Collins 2013) and *L. reuteri* (Lubkowicz et al. 2018) where the expression of these sensory proteins enabled the generation of AIP-I driven output signals when tested with all of the *S. aureus* subspecies in addition to other Gram-positive bacteria such as *S. epidermidis* and *B. subtilis* (Lubkowicz et al. 2018). The fluorescence readout observed with the pResponse strains has demonstrated the feasibility of this specially-devised bioengineering strategy in generating a staphylococcal targeting system using *S. aureus* QS metabolites as the main input or inducer biomolecules. The ability to design pathogen metabolite responsive genetic parts has enabled the development of bioengineered *S. epidermidis* as topical LBPs in treating *S. aureus* afflicted skin disease by which recombinant biotherapeutic proteins specifically filaggrin and penetrating peptide were expressed to restore human skin barriers (Dodds et al. 2020; Munivar & Whitfill 2015).

Conclusions

In this study, non-commensal *C. glutamicum* bacteria was successfully designed and tested as a potential staphylococcal responsive expression system. Using microbial engineering strategy, the bioengineered *C. glutamicum* harboring agrAC and RFP proteins showed AIP-I dependent expression when tested with the synthetic AIP peptide as well as *S. aureus* metabolites present in the culture supernatant. In summary, the findings attained from this study will provide a platform for future synthetic biological applications of *C. glutamicum* as a new staphylococcal targeting bioengineered chassis. This study therefore represents the first approach in developing bioengineered *C. glutamicum* as a staphylococcal targeting chassis using microbial bioengineering strategy.

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