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Design and in silico analysis of a whole-cell biosensor able to kill methicillin-resistant *Staphylococcus aureus*

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Abstract

The rise of methicillin-resistant *Staphylococcus aureus* (MRSA) infections has gained concern throughout the world over the past decades. Alternative therapeutic agents to antibiotics are rapidly growing to impede the proliferation of MRSA-caused infections. Lately, synthetic biology techniques have developed whole-cell biosensors by designing gene circuitry capable of sensing quorum-sensing (QS) molecules of pathogens and triggering expression of an antimicrobial moiety that kills MRSA and therefore prevents its further proliferation. Here, an *E. coli* was engineered in silico to act as a whole-cell biosensor that senses QS molecules from MRSA and triggers the expression of a bacteriocin that kills MRSA. To achieve this functionality, biosensor and bacteriocin modules were constructed and assembled into a vector. Both modules were codon-optimized to increase the yield production of the recombinant proteins. We then demonstrate in silico that the construction of a dual biosensor-killer plasmid, which holds two genetical modules known as biosensor and bacteriocin modules, enables the recombinant host to sense QS molecules from MRSA. Our designed whole-cell biosensor demonstrates in silico its ability to produce and secrete the bacteriocin as a function of the external concentration of autoinducer peptide from MRSA. These in silico results unravel the possibility of designing antimicrobial smarter therapeutics against resistant pathogens.

KEYWORDS

Escherichia coli, lacticin Q, MRSA, quorum-sensing, whole-cell biosensor

1 | INTRODUCTION

Antimicrobial resistant (AMR) infections will represent a serious threat by 2050 since it is estimated that 10 million people will annually die by that year if severe actions are not taken towards the management of antibiotics.[1] In 2017, the World Health Organization established that an effective therapeutic agent must be found with high priority to

Abbreviations: AIP, autoinducer peptide; AMR, antimicrobial resistance; *E. coli*, *Escherichia coli*; DNA, deoxyribonucleic acid; GFP, green fluorescent protein; KEGG, Kyoto Encyclopedia Genes and Genomes database; LAB, lactic acid bacteria; LnqQ, lacticin Q; MRSA, methicillin-resistant *S. aureus*; QS, quorum sensing; *S. aureus*, *Staphylococcus aureus*; SBP, Massachusetts Institute of Technology's Registry of Standard Biological Parts database.

neutralize AMR-pathogens including methicillin-resistant *Staphylococcus aureus* (MRSA).[2] The world prevalence for MRSA differs by region, but it has been estimated that 13–74% of worldwide infections are caused by this strain.[3] The rise of alternatives therapies to combat AMR infections was an immediate response after the fall of the golden age of discovery in antibiotics. The most current alternatives are vaccines, probiotics, prebiotics, phage therapy, endolysins, exolysins, living predatory microorganisms, and bacteriocins.[4]

Synthetic biology has also contributed towards the solution of this problem by engineering whole living cells to act as biosensor[5] and detect signal molecules known as quorum-sensing (QS) molecules secreted by pathogens.[6] QS is a natural cell-to-cell communication system that depends on the cell density in the cultures. While growing, bacterial cultures can produce, secrete, accumulate, and sense external signaling molecules, known as autoinducers, these are produced at basal levels but can also accumulate in the external medium. When the cell density of bacteria and autoinducer concentration are large enough to overpass a threshold level, the cell culture might change its phenotypic behavior, and this phenotypic response to QS would depend on the bacteria population size.[7,8] QS can happen in both Gram-positive and Gram-negative bacteria. Some QS-based phenotypical behaviors in bacteria are sporulation formation, virulence factors related to invasion, bioluminescence, virulence, among others. The QS-signaling molecules secreted by *Staphylococcus aureus* (*S. aureus*) are known as autoinducer peptides (AIP), which are produced by AgrC/AgrA operon system and they related to virulence factors.[9]

Several QS-based whole-cell biosensors have been previously developed, including an *Escherichia coli* (*E. coli*) enabled to identify *Vibrio cholerae* by detecting their QS molecules.[10] and a *Lactobacillus reuteri* that detects QS molecules from *S. aureus*.[11] Other works have improved the whole-cell biosensor features by enabling them to produce and secrete bacteriocins capable of killing other microorganisms,[12] even multiresistant microorganisms.[13]

Bacteriocins are short peptides which are exclusively produced by bacteria with the ability to kill or inhibit other bacteria. Bacteriocins differ in many ways from antibiotics because they are ribosomally produced while antibiotics are synthesized as secondary metabolites. In addition, the biological activity spectra of bacteriocins are narrow compared to the broad spectrum of antibiotics. However, bacteriocins tend to have a higher activity, with active ranges between the nano- to micromolar scale, meanwhile antibiotics have their optimal ranges within the micro- and milimolar range.[14] Moreover, due to their proteinaceous nature, bacteriocins can be modified by genetic engineering techniques.[15] Lactic acid bacteria (LAB) are the most reported bacteriocin producers in the world.[16] The bacteriocin lacticin Q (LnqQ) is naturally produced by *Lactococcus lactis*[17] and has a well-described activity against *S. aureus*.[18–20] The native production, secretion, and self-immunity of LnqQ is controlled by the genetical cluster *lnqQBCEDF*.[21,22]

Highlights

- Designing circuitry capable of sensing Quorum-Sensing (QS) molecules of pathogens and triggering expression of an antimicrobial moiety that kills multidrug-resistant bacteria.
- Dual Biosensor holds two genetic modules known as biosensor and bacteriocin modules, and they enable the recombinant host to sense QS-molecules from MRSA.
- Whole-cell biosensor shows, in silico, the potential to produce and secrete Lacticin Q in function to the external concentration of AIP from MRSA.

Here, we present an in silico designed expression vector in *E. coli* enabled to sense QS-signaling molecules from an MRSA and trigger the expression of a bacteriocin against MRSA in a concentration-dependent fashion. We performed a DNA optimization among the recombinant protein sequences to increase yield production in *E. coli*. We predicted the biosensor specificity by calculating a cross-talking behavior with other QS molecules classes. Finally, we predicted the solubility of the heterologous-produced proteins, its subcellular location in *E. coli*, the presence of B-cell epitopes in the recombinant bacteriocin and its protein allergenicity. The results of this project will have an impact on the future constructs of probiotic *E. coli* biosensors capable of detecting and kill MRSA cells. The design and theoretical work here presented contribute towards the construction of a whole-cell biosensor capable of self-regulating its own growth based on the available concentration of the QS-signaling molecules, thus avoiding the leaking production of bacteriocins which may contribute to the development of resistance in pathogens.

2 | MATERIALS AND METHODS

2.1 | Bacterial strain and sequences

E. coli BL21 (DE3) was used as biological chassis for overproduction of recombinant bacteriocins.[23] To design the QS biosensor, the annotated genome data from an

MRSA strain, the *S. aureus* strain N315, was used since they are one of the main causes of community-acquired and hospital-acquired methicillin-resistant infections.[24] For the bacteriocin module, we used the genetical cluster data for the structural, secretion, and self-immunity of the genes for the production of LnqQ from *L. lactis* strain QU5.[18,21] Below is presented the design with the elements of the QS biosensor module and the bacteriocin module as well as the general strategy to assemble the biosensor and bacteriocin modules with an expression vector.

2.2 | Design of the QS biosensor and bacteriocin modules

The biosensor module was designed to detect the external QS-signaling molecules from the MRSA strain, meanwhile the bacteriocin module contained the bacteriocin. The modules were plotted as follows: promoter-RBS-gene-double terminator. The ribosomal binding site (SBP no. BBa_B0034) and the double-terminator (SBP no. BBa_B0015) were downloaded from the Massachusetts Institute of Technology's Registry of Standard Biological Parts (http://parts.igem.org).[25] First, for the biosensor module, we used the *E. coli* constitutive promoter J23110 (SBP no. BBa_J23110) as the transcriptional regulator of the biosensor module genes *agrC* and *agrA*. We used the nucleotide and residues data for *agrC* (1,116 nucleotides and 371 residues, respectively. KEGG no. SA1843) and *agrA* (717 nucleotides and 238 residues, respectively. KEGG no. SA1844) from the MRSA which were downloaded from Kyoto Encyclopedia Genes and Genomes (KEGG) database (https://www.genome.jp/kegg/).[26] Second, for the bacteriocin module, we set the P2 promoter of the MRSA to regulate the expression of *lnqQBCEDF* and the *gfp* gene. The sequence for this promoter was not available, therefore, located it in the genome data file (GenBank no. BA000018.3). It is known that the loci of promoters are evolutionarily conserved between related species when located in the same strand.[27] We used the above premise to find the P2 promoter of the MRSA strain by two approaches: The first approach was to find this promoter by using the nucleotide sequence of *agrB* from the MRSA (564 nucleotides and 187 residues, KEGG no. SA1842) as reference since this gene is immediately downstream to P2 promoter. The second approach was done with the P2 promoter of *S. aureus* NCTC 8325.[28,29] because the genome data for strain is well-recognized as a bioinformatic reference.[30,31] By taking the best of both approaches, we found the structural composition and length of the P2 promoter of the MRSA. Finally, for the structural section of the bacteriocin module, we searched a data file containing the structural, secretion, and self-immunity genes of the cluster of *lnqQBCDEF* (GenBank no. AB712393.1),[21] which were downloaded from the GenBank database while the nucleotide and residues data from Green Fluorescent Protein (GFP) were downloaded from FPBase database, derived from *Aequorea victoria*.[34]

2.3 | General strategy to assemble the biosensor and bacteriocin modules

Template plasmids were downloaded from the AddGene repository (no. 116850), which were used for in silico construction of the biosensor and bacteriocin modules within this plasmid hereafter known as dual biosensor-killer plasmid. The in silico construction of the modules and the expression vector were designed using SnapGene software.

2.3.1 | Codon usage bias analysis of the native type and the optimized synthetic genes

Codon optimization of all the sequences was performed with the GenSmart Codon Optimization tool (https://www.genscript.com/tools/gensmart-codon-optimization) version Beta 1.0. Then, we used the CAIcal tool (http://genomes.urv.es/CAIcal/) to calculate the Codon Adaptation Index (CAI) from the sequences before and after the DNA optimization. This tool uses the protein sequence and the codon usage bias of native and the heterologous organism as inputs to perform the optimization. According to the author, the CAI ranges go from 0 to 1, being 1 if the query gene uses the most frequently used codons of the producer host.[36] Therefore, we used the Codon Usage Database (https://www.kazusa.or.jp/codon/)[37] to download the codon usage bias of *E. coli* B (Codon Usage Database no. 413997), *S. aureus* N315 (Codon Usage Database no. 158879), *L. lactis* subsp. *cremoris* (Codon Usage Database no. 1359), and *A. victoria* (Codon Usage Database no. 6100).

2.3.2 | Biosensor specificity by cross-talking prediction

A local pairwise sequence alignment of all the amino acidic of major AgrD sequence classes against the AgrD from MRSA strain was performed with EMBOSS Water (https://www.ebi.ac.uk/Tools/psa/emboss_water/) tool.[38] Four major representatives AgrD sequences were used as templates for the alignment,[39] and the annotated AgrD

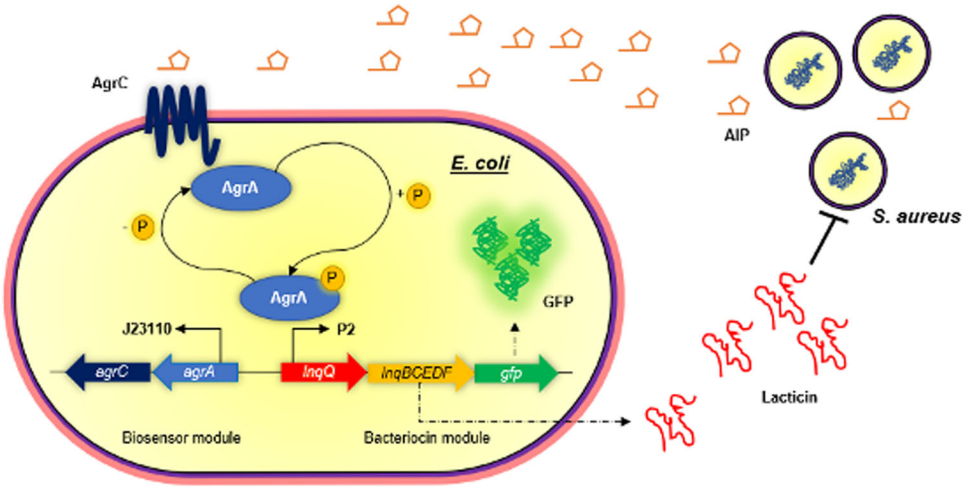

FIGURE 1 QS System of the sense-and-kill engineered *E. coli* with *S. aureus*. The biosensor module is regulated by the constitutive J23110 promoter (*agrC* and *agrA*). Detection of AIP-II is mediated by AgrC which activates the phosphorylation of AgrA and turns on the expression of bacteriocin module (*lnqQ* and *gfp*) regulated by P2 promoter

sequence of the MRSA strain were retrieved (KEGG no. SAS066).

2.3.3 | Prediction of protein solubility

Protein solubilization of all the proteins upon expression in *E. coli* was predicted by Protein-Sol (https://protein-sol.manchester.ac.uk/).[40] The overall accuracy of the tool was estimated at 70.6%. According to the Protein-Sol tool, any solubility value greater than 0.45 is considered to have enough solubility. Therefore, any protein lower than this value is predicted to have low solubility. This tool is unable to detect solubility for predicted membrane proteins.

2.4 | Prediction of subcellular location of proteins

We used BUSCA – Bologna Unified Subcellular Component Annotator (http://busca.biocomp.unibo.it), which is a web server tool that complies with other bioinformatic tools. This tool predicts the specific subcellular localization of a protein. This tool can discriminate between globular and membrane proteins.[41] We set the "Prokarya – Gram-negative" as the taxonomic origin of the input sequence because the protein will be produced in *E. coli*.

2.5 | Prediction of B-cell epitopes

BediPrep 2.0 and Discotope 2.0 were used to calculate the location of B-cell epitopes of the proteins by analyzing their lineal and three-dimensional structures. We used the entry data of LnqQ from Protein Data Bank (PDB no. 2N8P) as input for both applications. BediPrep 2.0 (http://www.cbs.dtu.dk/services/BepiPred/) was exerted to predict the location of B-cell epitopes in lineal sequences. This tool was trained with the Random Forest algorithm that was trained with epitopes and nonepitope amino acids to predict if a given amino acid residue is part of an epitope.[42] Meanwhile, DiscoTope 2.0 server (http://www.cbs.dtu.dk/services/DiscoTope/) was applied to predict discontinuous B-cell epitopes in proteins with reported three-dimensional structure.[43]

2.6 | Prediction of protein allergenicity

Allergenicity was evaluated by AllerCatPro version 1.7 (https://allercatpro.bii.a-star.edu.sg/) which predicts an immunoglobulin (Ig) E-respiratory (Type I) allergy based in the lineal and the three-dimensional protein structure.[44]

3 | RESULTS AND DISCUSSION

This work focused on (Figure 1) designing in silico *E. coli* cells enabled to sense external AIP-molecules produced by native QS-system of nearby MRSA cells. Briefly, engineered *E. coli* cells secrete fully active lacticin Q in function to the external concentration of AIP molecules. To achieve this goal, we selected *E. coli* cells as a whole-cell biosensor because they have been previously reported as QS-biosensor,[45] they are the world-preferred chassis to produce bacteriocins[23] and if the native operon of the selected bacteriocin is retained, transformed *E. coli* are enabled to secrete biologically active Gram-positive

TABLE 1 Codon usage analysis of the native and the optimized gene for expression in *E. coli*. The codon adaptation index (CAI) and the GC% content adjustment (GC%). The table is divided into two sections: Before and after optimization. The Gene section represents the name of the query and length means for the total nucleotide length of each query. Results in the table represent the adaptiveness of the query sequences in an *E. coli* host. It was not possible to calculate the codon usage for the *gfp* before optimization since there is "insufficient information for computing CAI using this reference table (Codon Usage Database no. 6100)

Parameters		Before optimization		After optimization	
Gene	**Length (nt)**	**CAI**	**GC%**	**CAI**	**GC%**
agrC	1116	0.407	28.2	0.770	43.3
agrA	717	0.402	27.2	0.766	44.6
lnqQ	162	0.532	33.3	0.675	46.9
lnqB	240	0.525	22.5	0.735	40.0
lnqC	480	0.518	23.1	0.771	42.1
lnqE	1299	0.511	25.9	0.773	41.0
lnqD	678	0.503	30.5	0.777	43.1
lnqF	795	0.521	24.5	0.747	42.4
gfp	NA	NA	NA	0.759	49.1

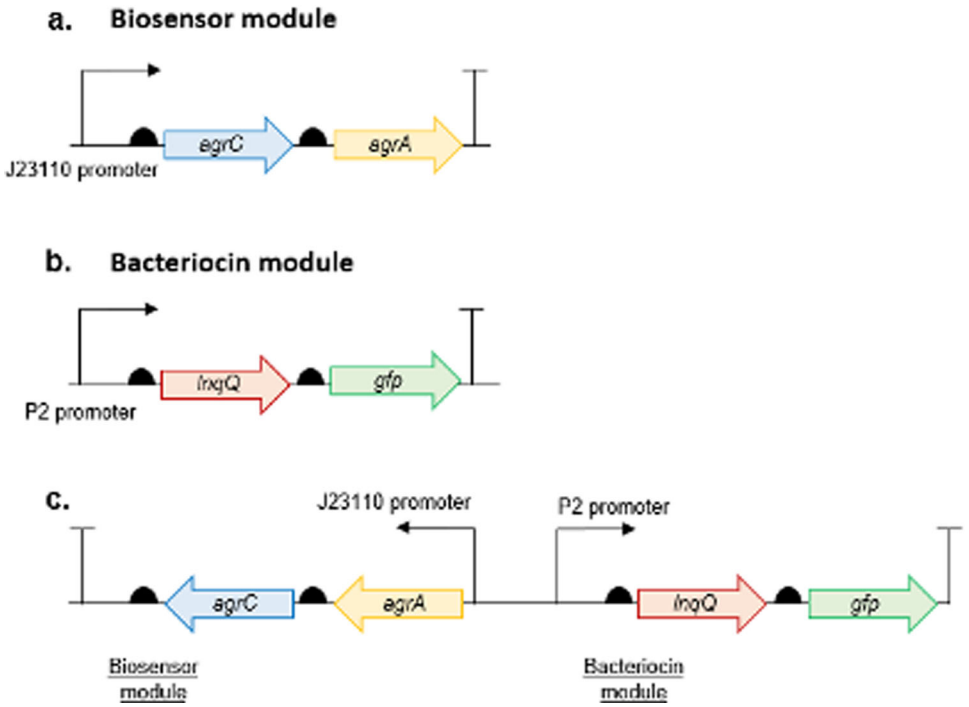

FIGURE 2 Biosensor and Bacteriocin modules. Biosensor module set with *agrC* and *agrA* transcriptionally regulated with J23110 promoter (A). Configuration of Bacteriocin module with *lnqQ* and *gfp* which is regulated by P2 promoter (B). The designing plot of the sensor-and-kill system with the back-to-back promoters, J23110 and P2 (C)

bacteriocins to the external milieu.[46] Our blueprint was designed to be applied in *E. coli* BL21 (DE3) because they have been successfully reported to produce and secrete IIa and IId class bacteriocins, being the latest important because they are the class where LnqQ belongs.[46]

To plot up the behavior of our in silico *E. coli* cell, it was necessary to design an expression vector with two genetical modules which we referred to as the biosensor module and bacteriocin module. Both modules were plotted as the following: promoter-RBS-structural gene-double terminator. The CAI and the %GC content of all the used protein sequences were substantially improved by web server tools. Also, and the typical restriction sites were removed (Table 1)

The principle of the design is next described: in the biosensor module, *agrC* and *agrA* are constitutively expressed (Figure 2A). AgrC can detect the presence

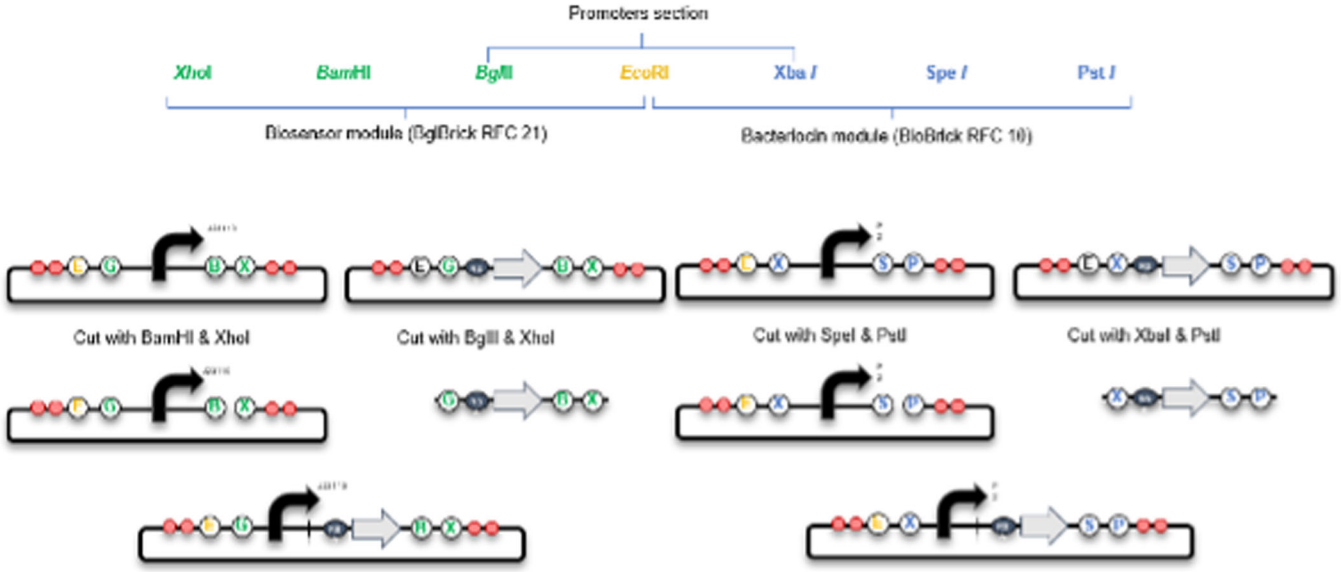

FIGURE 3 The in silico construction strategy. The biosensor module (A section) is constituted by BglBrick RFC21, bacteriocin module (C section) is represented by BioBrick RFC10 and the promoter's section (B section) was set in case promoters need to be changed. Each brick holds a prefix and suffix section, each one of them is enclosed by two double terminators (BBa_B0015; red circles) in both directions. The B section is bordered by BglII and XbaI (G-X) restriction sites with EcoRI (E) as neutral. The A section, EcoRI-BglII (E-G) restriction sites were placed as prefix and BamHI-XhoI (B-X) restriction sites were put as suffix. The restriction sites BglII and BamHI were used to settle the constitutive promoter J23110 and the structural section (RBS-gene) of the biosensor module genes. On the other hand, the C section, EcoRI-XbaI (E-X) restriction sites were set as prefix while SpeI-PstI (S-P) restriction sites were placed as suffix. The restriction sites XbaI and SpeI were used to build the inducible promoter P2 and the structural section (RBS-gene) of the bacteriocin module genes

TABLE 2 Cross-talking prediction. Protein alignment among AIP classes against AIP from MRSA. Alignment was performed in Clustal Omega while the identification (ID), similarity, gaps, score, and references (REF) were gathered from the EMOSS Water application

AIP class	Protein alignment	Length	ID	Similarity	Gaps	Score	Reference
I	MNTLFNLFFDFITGILKNIGNIAAYSTCDFIMDEVEVPKELTQLHE-	46	47.8%	65.2%	0.0%	17.00	[39,50]
II	MNTLVNMFFDFIIKLAKAIGIVGGVNACSSLFDEPKVPAELTNLYDK	47	100.0%	100.0%	0.0%	240.00	[39,50]
III	MKKLLNKVIELLVDFFNSIGYRAAYINCDFLLDEAEVPKELTQLHE-	46	32.6%	50.0%	0.0%	72.00	[39,50]
IV	MNTLYKSFFDFITGVLKNIGNVASYSTCYFIMDEVEIPKELTQLHE-	46	45.7%	60.9%	0.0%	105.0	[50]
MRSA	MNTLVNMFFDFIIKLAKAIGIVGGVNACSSLFDEPKVPAELTNLYDK	–	–	–	–	–	

of external AIP molecules once coupled to the *E. coli* membrane.[47] Then, AgrC suffers a conformational change and leads to the phosphorylation of the transcription factor AgrA. Next, the phosphorylated AgrA (AgrA-P) acts as a transcriptional factor of the P2 promoter.[29] Once the AgrA-P is coupled to the P2 promoter, the bacteriocin module is activated (Figure 2B)[47] and the structural genes (*lnqQ*) as well as the secretion and self-immunity genes (*lnqBCEDF*) for the *lnq* operon and the *gfp* are expressed. Finally, for the activity of the P2 promoter-controlled region, in response to the external AIP concentration, both modules (Figure 2C) were designed in a back-to-back fashion. We configured two template plasmids, each one containing individually the bacteriocin or the biosensor modules, respectively to be assembled (Figure 3) into a two-divergent module expression vector hereafter known as dual biosensor-killer (Figure 4).

S. aureus has four major classes of AIP (I to IV) molecules which differ in one or two residues in the mature AIP section of their amino acid sequence section.[39] It is known that AIP molecules may act as competitive inhibitors of QS among related strains. This biological phenomenon is known as cross-talking.[48] To predict the chance of cross-talking behavior in our biosensor, we used amino acid sequences of the four major AgrD classes and the annotated AgrD sequence of our MRSA strain to perform a local pairwise sequence alignment. According to the results (Table 2),

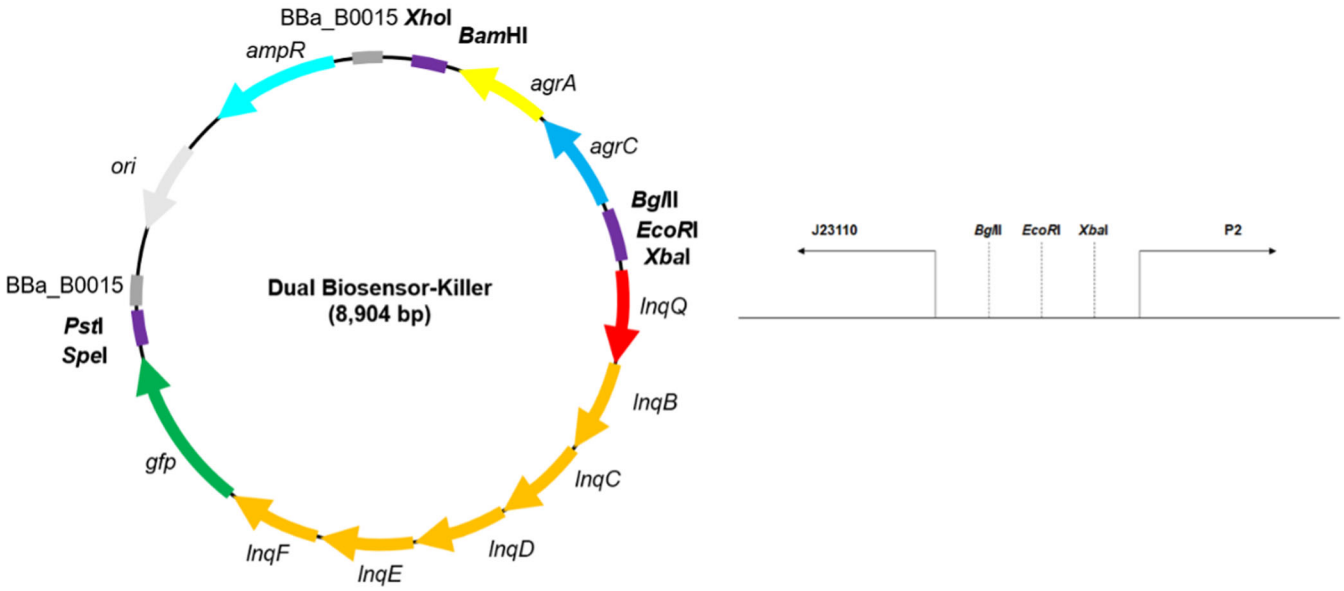

FIGURE 4 Schematic representation of the dual biosensor-killer plasmid. Biosensor module holds the *agrC* and *agrA* genes and is surrounded by EcoRI, BglII, BamHI, and XhoI restriction sites while the Bacteriocin module contains the genetical cluster *lnqQBCDEF* and *gfp* genes and is enclosed by EcoRI, XbaI, SpeI, and PstI. This plasmid has ampicillin resistance as marker selection feature and has a total length of 8904 bp

TABLE 3 Protein solubility by Protein-Sol tool

Query	Index	Solubility
AgrC	0.366	Invalid
AgrA	0.514	Soluble
LnqQ	0.655	Soluble
LnqB	0.655	Invalid
LnqC	0.644	Invalid
LnqD	0.388	Invalid
LnqE	0.609	Invalid
LnqF	0.607	Soluble
GFP	0.592	Soluble

TABLE 4 Prediction of subcellular location of proteins by BUSCA tool

Query	Score	Predicted location
AgrC	0.75	Plasma membrane
AgrA	0.7	Cytoplasm
LnqQ	0.7	Cytoplasm
LnqB	0.9	Plasma membrane
LnqC	0.83	Plasma membrane
LnqD	0.72	Plasma membrane
LnqE	0.86	Plasma membrane
LnqF	0.7	Cytoplasm
GFP	0.7	Cytoplasm

our biosensor was predicted to detect AIP-molecules class II.

We made an analysis of protein solubilization among all the used protein sequences to design the whole-cell biosensor since it can lead to high yield production.[49] Results (Table 3) indicated that LnqQ, LnqF, AgrA, and GFP were considered soluble, whereas the data for LnqB, LnqC, LnqD, and LnqE were considered invalid by the tool because they were membrane proteins. Then, we analyzed the predicted subcellular location of the heterologous proteins because is related to their functionality. Results (Table 4) indicated that AgrA, LnqQ, and LnqF were predicted in the cytoplasm, whereas AgrC, LnqB, LnqC, LnqD, and LnqE were located in the plasma membrane.

An important feature we wanted to find out was to determine whether the whole-cell biosensor along with LnqQ may develop allergenicity in humans since our goal for design this biosensor is to apply it in the future therapeutic agent. Therefore, it was essential to determine whether LnqQ had B-cell epitopes since allergenicity is highly determined by B- and T-cells lymphocytes. Our results (Table 5) showed that the lineal residues E13, S16-V19, and W21-D31 were above the epitope threshold (over 0.5) and no three-dimensional B-cell epitopes residues neither allergenicity sites were found. Part of the decision to build up the plot in function to *E. coli* was because they have been successfully reported as QS-biosensor to control an in vivo infection in nematodes and mice.[45]

TABLE 5 Prediction of B-Cell epitopes by Bediprep tool. In the epitopes section, if the position is over a threshold of 0.5, then, it is marked with an "E." structural section of the lineal sequence is predicted by NetsurfP and divided into three categories: H for helix. E for sheet, and C for coil. In the surface section, The surface section is divided into Burial (B) and Exposed (E) to predict the relative surface accessibility

Epitopes	..........E..EEEE.EEEEEEEEEE.....
Predictions	MAGFLKVVQLLAKYGSKAVQWAWANKGKILDWLNAGQAIDWVVSKIKQILGIK
Structural	CCHHHHHHHHHHHHCCHHHHHHHCCCCCHHHHHHHHHHHHHHHHHHHHCCC
Surface	EEEBEEBBEBBBEBEEEBBEBBBEEEEEBBEBBEBBBBEBBBEEBEEEEEEE

4 | CONCLUSION

In summary, we reported the design of an *E. coli* biosensor that can produce LnqQ based on the external concentration of AIP secreted by the MRSA. The response of this system is achieved by an expression vector that we designed as dual biosensor-killer. This plasmid can be used as a template for the construction of other biosensors with the ability to detect and kill pathogens. According to the in silico results, the construction is also enabled to sense and kill *S. aureus* strains that are AIP-II producers. Additionally, this strain is predicted to produce, secrete, and be self-immune to the production of lacticin Q, because we conserved the genetic cluster for Lacticin Q in this recombinant strain. Our predicted results indicated that LnqQ has enough solubility when overproduced. We also predicted no B-cell epitopes, either allergenicity. In this way, the LnqQ produced by this system seems to be safe to use as a potential therapeutic agent. Moreover, the in silico results here reported, open up the field to develop and design novel and dynamic antimicrobial agents, in the form of live engineered microorganisms, to treat both sensitive and resistant pathogens.

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CONFLICTS OF INTEREST

The authors declare no commercial or financial conflict of interests.

AUTHORS' CONTRIBUTIONS

Conceptualization and study design: DFBC, and JRMR; writing—original draft preparation: DFBC, FJBC, ALB, and JRMR; writing—review and editing: DFBC, FJBC, ALB, and JRMR; supervision: ALB, and JMR. All authors reviewed the final version of the article and approved the submitted version.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The datasets employed in this study do not require ethical approval.

AVAILABILITY

All data generated during this study are included in this article. The simulated data generated including in silico designed plasmid will be electronically shared by the corresponding author upon authorization.

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