

A RECOMBINANT EXPRESSION SYSTEM THAT SENSES PATHOGENIC MICROORGANISMS

Field of the invention

The invention lies in the field of recombinant expression systems designed for autonomous regulation of the expression of at least one peptide, for instance an antimicrobial peptide and use of such systems, e.g. for detection and/or treatment of infections such as gastrointestinal infections.

Background of the invention

Gastrointestinal infections are a serious cause of significant morbidity and mortality over the world each year (Culligan et al. 2009). Gastrointestinal infectious diseases are becoming increasingly challenging to treat mainly because of the increasing emergence of very dangerous antibiotic resistant microorganisms, also known as 'super bugs'. Further, no new antibiotic classes have been discovered in recent past and pharmaceutical companies have significantly reduced their investment in antimicrobial research (Alanis, 2005). Consequently, there is a critical need to explore and develop new and innovative therapeutics for instance against *Vibrio cholerae*.

Cholera is a very serious and highly infectious disease caused by *V. cholerae*, which infects the human GI tract through transmission by contaminated water and/or food. *Cholera*-infected patients suffer acute diarrhoea. If they are left untreated, they may die within a few hours. Today, *cholera* is still prevalent in many developing countries, with the highest rates in Asia (Sack et al, 2006).

Conventional methods of treating *cholera* include oral rehydration therapy, antibiotic therapy and vaccines. Oral rehydration and antibiotics have been effective in treating cholera, but antibiotic resistant strains are becoming more common (Sack et al. 2006; Shukla et al. 2008). Therefore, antibiotics should not be used as a preventative measure, because this will encourage the spread of resistant strains. Moreover, while oral rehydration therapy is an effective treatment method, it cannot prevent infection. In addition, although two *cholera* vaccines are available, the vaccines only provide around 50-60% immunity in the first two years (Sinclair et al. 2011). Preventing people from being infected in a *cholera* epidemic is important, as it will reduce the likelihood of healthy people being infected and further contaminating the environment and other people.

Thus, there remains a need in the art for novel, unconventional antimicrobial strategies – for instance against *V. cholerae* – that can complement current antibiotic therapy, but could also be used as a preventative measure.

5 Summary of the Invention

The inventors of the present invention have surprisingly found that the above-formulated need can be met by a recombinant expression system comprising at least:

- (i) a first nucleotide sequence encoding for at least one protein of a quorum sensing system capable of detecting the presence, amount or both of a microorganism of interest by forming a complex with a marker molecule indicating the presence of said microorganism;
 - (ii) a second nucleotide sequence encoding for at least one antimicrobial peptide, wherein the at least one antimicrobial peptide is effective against the microorganism of interest detected by the at least one protein encoded by the first nucleotide sequence,
 - 15 (iii) a third nucleotide sequence encoding for a genetic inverter that inhibits expression of the second nucleotide sequence,
- wherein the genetic inverter is under control of an inducible promoter and wherein the inducible promoter is induced if the complex of the at least one protein encoded by the first nucleotide sequence and the marker molecule indicating the presence of said microorganism is below a threshold concentration and is not induced if the complex of the at least one protein encoded by the first nucleotide sequence and the marker molecule indicating the presence of said microorganism exceeds a threshold concentration.

- 25 A second aspect of the invention includes a recombinant cell comprising a recombinant expression system according to the invention.

- A third aspect of the invention includes a method of sensing and killing pathogenic microorganisms, the method comprising contacting the recombinant cell of the invention with the pathogenic microorganism.

In a fourth aspect, the invention is directed at the use of a recombinant expression system comprising at least:

- (i) a first nucleotide sequence encoding for at least one protein of a quorum sensing system capable of detecting the presence, amount or both of a microorganism of

interest by forming a complex with a marker molecule indicating the presence of said microorganism;

(ii) a second nucleotide sequence encoding for at least one detectable marker,

(iii) a third nucleotide sequence encoding for a genetic inverter that inhibits expression

5 of the second nucleotide sequence,

wherein the genetic inverter is under control of an inducible promoter and

wherein the inducible promoter is induced if the complex of the at least one protein encoded by the first nucleotide sequence and the marker molecule indicating the presence of said microorganism is below a threshold concentration and is not induced

10 if the complex of the at least one protein encoded by the first nucleotide sequence and the marker molecule indicating the presence of said microorganism exceeds a threshold concentration,

for detection of at least one type of microorganism.

15 Further embodiments will be apparent to a person skilled in the art with reference to the following description of various non-limiting embodiments.

Brief description of the drawings

The drawings are not necessarily drawn to scale, emphasis instead generally being
20 placed upon illustrating the principles of various embodiments. In the following description, various embodiments of the invention are described with reference to the following drawings.

Figure 1A:

25 Overview of a recombinant expression system - exemplified with *V. cholerae* - and the state of the individual components, if the marker molecule produced by the microorganism is below a threshold concentration.

In the exemplary case of *V. cholerae*, an *E. coli* Nissle strain was engineered to
30 express an antimicrobial peptide and the CqsS/CAI-1 quorum-sensing phosphorelay system of *V. cholerae*. In this system (S)-3-hydroxytridecan-4-one (CAI-1) is the marker molecule and CqsS – the CAI-1 receptor – is part of the first nucleotide sequence. At low cell density of *V. cholerae*, when the concentration of the marker molecule CAI-1 is below the detection limit, i.e. the threshold, CqsS functions as a kinase. Following auto-
35 phosphorylation at His194, the phosphoryl group is transferred to Asp618 on the CqsS receiver domain. The next transfer is to His58 on LuxU. LuxU, in turn, transfers the

phosphoryl group to Asp47 on LuxO. Once phosphorylated, LuxO binds to σ^{54} factor. This complex binds to Qrr4 promoter (pQrr4), which is the inducible promoter controlling the genetic inverter. The genetic inverter in turn negatively regulates expression of the antimicrobial peptide.

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Thus, at low cell density of *V. cholerae* production of the antimicrobial peptide is repressed and rapidly started upon CAI-1 detection.

Figure 1B Overview of a recombinant expression system - exemplified with *V. cholerae* - and the state of the individual components, if the marker molecule produced by the microorganism of interest is exceeding a threshold concentration. At high cell density of *V. cholerae*, the marker molecule CAI-1 accumulates and binds CqsS, and switches CqsS to a phosphatase. Phospho-flow is reversed and LuxO is dephosphorylated. The genetic inverter is not produced, resulting in the synthesis of the antimicrobial peptide, which kills *V. cholerae*.

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Figure 2 Overview of different types of genetic inverters

Depicted are three different genetic inverters types

The first genetic inverter is based on a promoter-repressor pair as exemplified with the HapR repressor, which blocks transcription from the pAhpA promoter and hence of any antimicrobial peptide under control of the pAhpA promoter. The pAhpA promoter labelled pAp (promoter of Antibacterial peptide) is an example for a promoter driving the antimicrobial peptide.

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The second genetic inverter is based on a sRNA. In this case, the genetic inverter sRNA binds to the mRNA of the antimicrobial peptide and this represses translation of the antimicrobial peptide.

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The third genetic inverter is a CRISPRi based genetic inverter. It is based on the dCas9 protein and associated guide RNA (gRNA). These in tandem repress expression of any given promoter by blocking the transcription initiation.

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Figure 3 Overview of plasmid maps of an exemplary recombinant expression system
Shown is the plasmid design of an exemplary recombinant expression system according to the present invention. In this example a recombinant expression system comprising a CRISPRi based genetic inverter is depicted.

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Figure 4 Schematic overview of the working principle of a recombinant expression system comprising a promoter-repressor type genetic inverter, here exemplified with pAphA and HapR.

In order to characterize the pAhpA promoter – HapR repressor pair, a single plasmid, in which Red Fluorescent Protein (RFP) is expressed under control of the pAhpA promoter, was constructed. Using this system, the level of RFP fluorescence of the culture is controlled by addition of different levels of arabinose. When no arabinose is present, the pBAD promoter is not induced and no Qrr (sRNA preventing HapR translation) is produced, thereby allowing the expression of the HapR protein, which in turn represses the expression from the pAphA promoter. Therefore, no RFP is expressed in the absence of arabinose. As soon as the arabinose concentration exceeds a threshold level, HapR expression is inhibited, thereby allowing transcription from the pAphA promoter and thus RFP expression.

Figure 5 Shown is a comparison of fluorescence levels of cultures expressing HapR following induction by arabinose and cultures not expressing HapR. HapRs coming from two different serotypes of *V. cholerae*, 01 and 037 strains, were compared.

Figure 6 Shown are differences in GFP expression level with different Atc and IPTG concentrations in the medium in cultures expressing a CRISPRi based genetic inverter. The genetic inverter, with which these results were generated, utilizes the CRISPRi system, in which the dCas9 (dead Cas9) protein in tandem with corresponding gRNA effectively represses expression from a promoter. Here the dCAS9 protein was under control of the ATc induced promoter and the gRNA under control of an IPTG induced promoter. The results demonstrate the CRISPRi genetic inverter has a good noise to signal level ratio.

Figure 7 Shown are the plasmid maps for an exemplary recombinant expression system comprising two nucleic acid constructs. The first nucleic acid construct, i.e. the first plasmid, harbours the CqsS, and LuxU as well as LuxO under pBAD promoter control, while GFP under pQrr4 promoter control is located on the second plasmid.

Figure 8 and Figure 9 show that depending on different CAI-1 concentrations GFP expression level varies, demonstrating that the recombinant expression system of Figure 7 was able to sense the presence of CAI-1 marker molecule in the medium (the

GFP fluorescence of the medium is a function of CAI-1 concentration). In the test construct the pBAD promoter drives LuxO. Hence, at high arabinose concentrations the pBAD promoter is induced and LuxO is expressed. When CAI-1 is added to the set-up, a drop in the GFP expression was observed. This is due to the downregulation of pQrr4 promoter by the CqsS cascade originating from *V. cholera*. In contrast, no alteration of GFP expression was observed when arabinose was present, but CAI-1 was absent. This shows the system is able to sense the presence of CAI-1 and represses the expression of GFP. The system is inducible by arabinose in order to add an additional level of control.

Figure 10 An overview is given over five different plasmids constructs that were generated to evaluate the activity of Microcin S (mcsS) and Microcin immunity protein (mcsI) in *E. coli* Top10 cells.

Figure 11 Shown are growth curves of *E. coli* Top10 with different plasmids.

(A) p000k is an empty pBbE8K vector containing just the pBAD promoter without any coding sequence. Induction with L- Arabinose did not show significant impact on the growth of the cells. (B) pBbE8K + McsS-YebF is a vector containing McsS-YebF fusion peptide insert under control of the L-arabinose-induced araC pBAD activator-promoter. When induced with arabinose, a complete growth inhibition of the host cell (*E. coli* Top10) was observed, while un-induced cultures did not show any growth reduction. (C) pBbE8K + McsI + McsS-YebF is a vector containing McsS-YebF fusion peptide insert and McsI is produced constitutively under *rrnB* promoter. When induced, McsI (immunity protein) is able to restore the growth of *E. coli* by proteolysing the Microcin peptides. (D) pBbE8K + McsS is a vector containing the McsS insert under control of the L-arabinose-induced araC PBAD activator-promoter. Similarly, when induced with arabinose there is a complete growth inhibition of the host cell (*E. coli* Top10), while un-induced cultures did not show any growth reduction. Also the introduction of McsI gene restored the growth of *E. coli* cells.

Figure 12 Shown is the effect of Microcin S (construct 5 of Figure 10) on *E. coli* cells.

Figure 13 Shown are the results of experiments related to expression and purification of Microcin S. (A) Quant-it assay standard curve, calibrated at measuring the fluorescence at 470/570 nm. (B) SDS PAGE results, Lane 1: Microcin S induced by 0.125% arabinose and Lane 2: Microcin S uninduced

Figure 14 Characterization of peptide the antimicrobial peptide CM11 (P3) expression in *E. coli* Top10. P3 after induction with 0.2% Ara reduces the growth of *E. coli* (bottom line) and uninduced sample grows normally (top line).

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Figure 15 Characterization of Lysis E7 expression in *E. coli*. Lysis E7 expressed when 200nM of atc (anhydrotetracycline) is able to lyse *E. coli* within 4hrs (bottom line). Uninduced sample grows normally (top line).

10 **Figure 16** MIC of the synthetic peptide CM11 (P3) against *V. cholerae* (A1522 Rugose-GFP). The target cells were effectively killed at an MIC of 50µM. As a control only DMSO was used. As the target cells express GFP embedded into its chromosome, the GFP fluorescence (B) along with the absorbance (growth) (A) was monitored in this experiment.

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Figure 17 MIC of the synthetic peptide CM11 (P3) against *E. coli* Nissile. The target cells were susceptible to the peptide at an MIC of 25µM. As a control only DMSO was used.

20 Detailed Description

A new recombinant expression systems designed for autonomous expression of at least one peptide is disclosed.

25 Thus, in a first aspect the invention relates to a recombinant expression system comprising at least:

(i) a first nucleotide sequence encoding for at least one protein of a quorum sensing system capable of detecting the presence, amount or both of a microorganism of interest by forming a complex with a marker molecule indicating the presence of said
30 microorganism;

(ii) a second nucleotide sequence encoding for at least one antimicrobial peptide, wherein the at least one antimicrobial peptide is effective against the microorganism of interest detected by the at least one protein encoded by the first nucleotide sequence,

(iii) a third nucleotide sequence encoding for a genetic inverter that inhibits expression
35 of the second nucleotide sequence,

wherein the genetic inverter is under control of an inducible promoter and

wherein the inducible promoter is induced if the complex of the at least one protein encoded by the first nucleotide sequence and the marker molecule indicating the presence of said microorganism is below a threshold concentration and is not induced if the complex of the at least one protein encoded by the first nucleotide sequence and the marker molecule indicating the presence of said microorganism exceeds a threshold concentration

"One or more", as used herein relates to at least 1 and includes 1, 2, 3, 4, 5, 6, 7, 8, 9 and more. Any function assigned to the "one or more" species may be achieved independently by each of the species or achieved by the combination of the more than one species.

The term "recombinant expression system" as used herein refers to a combination of an expression vector, its cloned nucleic acid, and optionally the host for the vector that provide a context to allow for the production of the sequence encoded by a nucleic acid at a high level in the host.

The recombinant expression system may be designed to operate *in vitro* or may be a cell based recombinant expression system. In a preferred embodiment, the recombinant expression system is a prokaryotic cell based system.

The term "nucleic acid construct" as used herein refers to a nucleic acid molecule that includes a given sequence. The nucleic acid may be DNA, RNA, DNA:RNA hybrids, PNA and the like, but preferably is DNA. The construct can be an expression vector for expression of a protein encoded by a recombinant gene carried by said vector, a plasmid, cosmid, or artificial chromosome. A preferred vector is a vector that can self-replicate and express a given nucleic acid sequence included therein.

Quorum sensing is the regulation of gene expression in response to fluctuations in cell-population density. Quorum sensing bacteria (Gram-positive and Gram-negative) produce and release chemical signal molecules called autoinducers that increase in concentration as a function of cell density. The detection of a minimal threshold stimulatory concentration of an autoinducer leads to an alteration in gene expression.

Thus, the protein of a quorum sensing system may be any protein of a quorum sensing signalling cascade capable of detecting the presence, amount or both of a

microorganism of interest by forming a complex with a marker molecule indicating the presence of said microorganism.

As used herein the term "microorganism" is intended to include living cellular organisms, both unicellular and multicellular that are less than 5 mm in length, and include but are not limited to bacteria, fungi, archaea, protists, green algae, plankton, planarian, amoebas and yeasts, or spores formed by any of these.

The term "antimicrobial peptide" refers to a peptide that exhibits microbicidal or microbiostatic and/or bactericidal or bacteriostatic properties that enables the peptide to kill, destroy, inactivate, or neutralize a microorganism; or to prevent or reduce the growth, ability to survive, or propagation of a microorganism.

The term "genetic inverter" as used herein refers to a regulatory element that inhibits expression of the at least one antimicrobial peptide or the at least one detectable marker, thereby preventing that the at least one antimicrobial peptide is active in the absence of the microorganism of interest or that the at least one detectable marker can be detected in the absence of the microorganism of interest.

As used herein, the terms "promoter", "promoter element", and "promoter sequence" refer to a DNA sequence, which is capable of controlling the transcription of the nucleotide sequence into mRNA when the promoter is placed at the 5' end of (i.e., precedes) a nucleotide sequence. Thus, a promoter is typically located 5' (i.e., upstream) of a nucleotide sequence whose transcription into mRNA it controls, and provides a site for specific binding by RNA polymerase and for initiation of transcription.

The term "inducible promoter" as used herein refers to a promoter that can be regulated by the presence or absence of biotic or abiotic factors such as transcription factors. Thus, an inducible promoter can either be positively or negatively regulated. For instance an inducible promoter can mean a promoter that has low activity in the absence of an activator. On the contrary, a promoter can have high activity in the absence of an agent, which negatively regulates the promoter.

In this regard it should be noted that in the inventive recombinant expression system the inducible promoter positively regulates the genetic inverter. Thus, the genetic inverter is expressed whenever the promoter is active and can then negatively regulate

expression of the at least one antimicrobial peptide or the at least one detectable marker. In other words, the inducible promoter is downregulated upon presence of the complex formed by the at least one protein of a quorum sensing system capable of detecting the presence, amount or both of a microorganism of interest and the marker molecule indicating the presence of the microorganism of interest. Consequently, the genetic inverter is also downregulated and not expressed once this complex is present in sufficient quantities, which leads to expression of the at least one antimicrobial peptide or the at least one detectable marker.

The term "threshold" as used herein refers to the minimum level at which the complex of the at least one protein encoded by the first nucleotide sequence and the marker molecule indicating the presence of the microorganism of interest induces the inducible promoter.

The term "marker molecule" as used herein refers to any molecule that can form a complex with the at least one protein of a quorum sensing system encoded by the first nucleotide sequence.

In various embodiments of the recombinant expression system, the marker molecule is chosen from the group consisting of small molecules, lipids, phospholipids, amino acids, monoamines, peptide, proteins, glycoproteins, or gases.

The term "first nucleotide sequence", "second nucleotide sequence" etc., as used herein, relate to nucleic acid sequences that encode for one or more gene products of a given functionality. Accordingly, the first nucleotide sequence may comprise one or more genes with each of said genes encoding for a separate gene product. This applies similarly to other nucleotide sequences. All of these nucleotide sequences may comprise additional non-coding sequence stretches required for transcription and translation of the respective coding sequences.

Generally, the nucleic acid constructs may additionally comprise further regulatory elements, such as enhancers or silencers, all of which are well known to those skilled in the art.

Advantageously, this recombinant expression system is capable of autonomously regulating the expression of the at least one antimicrobial peptide. Thus, once the

concentration of the complex of the at least one protein of a quorum sensing system and the marker molecule indicating the present of the microorganism of interest is exceeding a threshold concentration, the genetic inverter is inactivated and the at least one antimicrobial peptide is produced.

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On the contrary, if the concentration of the complex of the at least one protein of a quorum sensing system and the marker molecule indicating the presence of the microorganism is below the threshold concentration, i.e., the microorganism of interest is not present or present in low amounts, the system autonomously represses production of the at least one antimicrobial peptide.

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Moreover, the combination of inducible promoter and genetic inverter also allows for a very precise control of the expression of the at least one antimicrobial peptide.

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Therefore, this recombinant expression system may be used as a preventative measure, since it will only produce the at least one antimicrobial peptide upon autonomous detection of the microorganism of interest.

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In various embodiments of the recombinant expression system the genetic inverter (a) comprises a nucleotide sequence encoding a repressor molecule and a promoter repressed by the repressor, with said promoter controlling expression of the second nucleotide sequence, (b) comprises a nucleotide sequence encoding an sRNA specific for the mRNA encoded by the second nucleotide sequence or (c) is a CRISPRi system comprising a nucleotide sequence encoding for a gRNA sequence specific for the second nucleotide sequence and a nucleotide sequence encoding for dCas9 protein

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As demonstrated in Figures 6, 8 and 9 these genetic inverters allow a precise and reliable repression of the antimicrobial peptide when the concentration of the complex of the at least one protein of a quorum sensing system and the marker molecule indicating the presence of the microorganism of interest is below the threshold concentration.

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By carefully choosing a suitable genetic inverter the recombinant expression system may be targeted to the specific needs of a given application, i.e. to targeting a variety of microorganisms.

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In a suitable promoter-repressor pair the repressor inhibits transcription from the promoter of the at least one antimicrobial peptide or the at least one detectable marker. Hence, the promoter-repressor pair represses transcription of the at least one antimicrobial peptide or the at least one detectable marker.

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Examples for promoter-repressor pairs are the pAhpA promoter and the HapR repressor., PhiF and pPhiF, LacI and pTac, TetR and pTetR and BetI with pBetI.

As used herein, the term "sRNA" refers to a short-length RNA (small RNA), which is usually 200 or less nucleotides in length, which is not translated into protein and which effectively inhibits the translation of a specific mRNA by complementary binding. Thus, an sRNA-based genetic inverter represses translation of the antimicrobial peptide.

A genetic inverter based on the CRISPRi system represses transcription of the antimicrobial peptide.

Based on the bacterial genetic immune system - CRISPR (clustered regularly interspaced palindromic repeats) pathway, the CRISPR interference (CRISPRi) technique is a genetic perturbation technique that allows for sequence-specific repression or activation of gene expression in prokaryotic and eukaryotic cells.

The CRISPRi system relies on the catalytically inactive Cas9 enzyme termed dCas9. dCas9 is unable to cleave dsDNA but retains the ability to target and bind DNA. Targeting specificity is determined by complementary base-pairing of a single guide RNA (gRNA) to the genomic loci. The gRNA is a chimeric noncoding RNA that can be subdivided into three regions: a 20 nt base-pairing sequence, a 42 nt dCas9-binding hairpin and a 40 nt terminator. Taken together gRNA and dCas9 provide a minimum system for gene-specific regulation in any organism.

When designing a synthetic gRNA, only the 20 nt base-pairing sequence is modified from the overall template.

Moreover, tailoring of the recombinant expression system may also be achieved via altering the first nucleotide sequence encoding for at least one protein of a quorum sensing system capable of detecting the presence, amount or both of a microorganism of interest by forming a complex with a marker molecule indicating the presence of said

microorganism. Via choosing a suitable protein of a quorum sensing system, it is possible to target a desired microorganism.

In one embodiment of the recombinant expression system the genetic inverter is a CRISPRi system, wherein the nucleotide sequence encoding for a gRNA sequence specific for the second nucleotide sequence is under control of the inducible promoter and the nucleotide sequence encoding for dCas9 is constitutively expressed.

As used herein the term "gRNA" refers to guided RNA, i.e. to a RNA, which is specific for the target DNA and can form a complex with dCas9 protein and bring dCas9 protein to the target DNA.

As demonstrated in Figure 6 such a recombinant expression system allows a precise and reliable repression or expression, respectively, of the peptide or protein of interest.

In a preferred embodiment of such a recombinant expression system the promoter controlling the genetic inverter sequence is the Qrr4 promoter (SEQ ID NO 4).

In one embodiment of the recombinant expression system the detection mechanism of the first nucleotide sequence is based on the CqsS/CAI-1 quorum-sensing phosphorelay system of *V. cholerae*.

The mechanism of the *V. cholerae* CqsS/CAI-1 quorum-sensing phosphorelay system is as follows: CqsS is the CAI-1 receptor. At low cell density when CAI-1 concentration is below the detection limit, CqsS functions as a kinase. Following auto-phosphorylation at His194, the phosphoryl group is transferred to Asp618 on the CqsS receiver domain. The next transfer is to His58 on LuxU. LuxU, in turn, transfers the phosphoryl group to Asp47 on LuxO. Once phosphorylated, LuxO via binding to σ 54 factor activates the transcription from the Qrr4 promoter (pQrr4). At high cell density CAI-1 accumulates, binds CqsS, and switches CqsS to a phosphatase. Phospho-flow is reversed and LuxO is dephosphorylated. Consequently, transcription from the Qrr4 promoter ceases.

It is preferred that the detection mechanism of the first nucleotide sequence is or comprises a heterologous detection system. This may help to avoid interference with cellular pathways of the host cell.

In one embodiment of the recombinant expression system the first nucleotide sequence encodes for the proteins of the (S)-3-hydroxytridecan-4-one (CAI-1) sensor module and thus comprises the nucleotide sequence encoding for the LuxO protein having the amino acid sequence as set forth in SEQ ID NO: 8, the nucleotide sequence encoding for the CqsS protein having the amino acid sequence as set forth in SEQ ID NO: 6 and the nucleotide sequence encoding for the LuxU protein having the amino acid sequence as set forth in SEQ ID NO: 7.

In various embodiments of the recombinant expression system the one or more antimicrobial peptide is selected from the group consisting of McsS (SEQ ID NO 9), G12.21 (SEQ ID NO 10), G14.15 (SEQ ID NO 11), CM11 (SEQ ID NO 12), CM15 (SEQ ID NO 13) and Magainin 2 (SEQ ID NO 14), L1 (SEQ ID NO 30), L2 (SEQ ID NO 31), B1 (SEQ ID NO 32) and CP1 (SEQ ID NO 33).

In one embodiment of the recombinant expression system the antimicrobial peptide is Microcin S (SEQ ID NO 9).

In various embodiments of the recombinant expression system the antimicrobial peptide is fused to a secretory peptide.

In one embodiment of the recombinant expression system the at least one antimicrobial peptide is fused to the YebF (SEQ ID NO 15) secretory peptide. Other possible secretory peptides include, FlgM (SEQ ID NO 34) or lysis E7 (SEQ ID NO 35).

In a preferred embodiment of the recombinant expression system the at least one antimicrobial peptide McsS (SEQ ID NO 9) is fused to a YebF peptide (McsS-YebF, SEQ ID NO 16).

In a further aspect the invention relates to a recombinant cell comprising a recombinant expression system as described above.

Such an embodiment has the advantage the cell can be used as preventative measure against a microorganism of interest and autonomously initiates expression of an antimicrobial peptide, if the concentration of this microorganism exceeds a threshold level.

In various embodiments of this aspect of the invention the recombinant cell is a genetically engineered prokaryotic cell.

- 5 In various embodiments the prokaryotic cell is a probiotic bacteria.

Such an embodiment has the advantage that the probiotic bacteria can be manipulated and grown easily and efficiently even in large-scale fermenters, thereby leading to substantial cost advantages.

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Moreover, engineered probiotic bacteria could be ingested in a similar manner as how existing probiotic bacteria are taken (e.g. yoghurts, drinks, capsules, dried forms, etc.), which can be made available in very affordable forms, readily distributed when necessary and taken without the need for medical expertise/devices (e.g. presence of
15 doctors, use of needles, etc.). These are important factors for use in developing countries where infections such as cholera are most prevalent.

In various embodiments the prokaryotic cell is an *Escherichia coli* cell.

- 20 In one embodiment the *Escherichia coli* cell is a probiotic *E.coli* Nissle cell.

These recombinant cells have the advantage that they can actively sense and kill the microorganism of interest such as a human pathogen, e.g. *V. cholerae*.

- 25 Advantageously, the inventive recombinant expression system allows the secretion of the antimicrobial peptide. Thus, there is no negative effect on the host cell, as it would be the case, if the host cell has to be destroyed or lysed in order to release the at least one antimicrobial peptide. This leads to a higher production of the antimicrobial peptide, since the host cell can continuously produce the antimicrobial peptide until the
30 concentration of the complex of the at least one protein of a quorum sensing system and the marker molecule indicating the present of said microorganism is below the threshold level.

- 35 However, it is also possible that the at least one antimicrobial peptide is released through lysis of the recombinant cell.

In a further aspect, the present invention relates to a method of sensing and killing pathogenic microorganisms, the method comprising contacting the recombinant cell described above with the pathogenic microorganism.

- 5 This method has the advantage that it is designed to protect from suffering from microorganisms such as *Vibrio cholerae*. It serves as a preventative measure, since it will only produce the antimicrobial peptide upon detection of the microorganisms such as *V. cholerae*. It could potentially be used as a drug.
- 10 One embodiment of the method of sensing and killing pathogenic microorganisms comprises administering the recombinant cell to a subject.

In one embodiment of the method of sensing and killing pathogenic microorganisms the pathogenic microorganism is a human pathogen.

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In a preferred embodiment of the method of sensing and killing pathogenic microorganisms the pathogenic microorganism is selected from the group consisting of *Pseudomonas aeruginosa*, *Clostridium difficile*, *Escherichia coli*, *Helicobacter pylori*, *Salmonella*, *Vibrio cholerae* and *Yersinia*.

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In a further aspect, the present invention relates to the use of a recombinant expression comprising at least:

- (i) a first nucleotide sequence encoding for at least one protein of a quorum sensing system capable of detecting the presence, amount or both of a microorganism of interest by forming a complex with a marker molecule indicating the presence of said microorganism;
- 25 (ii) a second nucleotide sequence encoding for at least one detectable marker,
- (iii) a third nucleotide sequence encoding for a genetic inverter that inhibits expression of the second nucleotide sequence,
- 30 wherein the genetic inverter is under control of an inducible promoter and wherein the inducible promoter is induced if the complex of the at least one protein encoded by the first nucleotide sequence and the marker molecule indicating the presence of said microorganism is below a threshold concentration and is not induced if the complex of the at least one protein encoded by the first nucleotide sequence and
- 35 the marker molecule indicating the presence of said microorganism exceeds a threshold concentration,

for detection of at least one type of microorganism.

Such a system has the advantage that it can autonomously detect the presence of a microorganism of interest.

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As used herein the term “detectable marker” refers to any organic chemical compound, peptide, protein or DNA or RNA molecule capable of giving rise to a detectable signal that indicates the presence of microorganism of interest. A “detectable signal” is a signal that is either visible to the naked eye or detectable (readable) with the aid of an instrument, such as a spectrophotometer. Accordingly, detection may be by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. Detection peptides are well known in the art for detection in immunoblotting, ELISA and other such assay techniques.

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Other embodiments are within the following claims and non-limiting examples. It is to be understood that these examples are for illustrative purposes only and are not to be construed to limit the scope of the present invention.

Examples

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Example 1: Construction of a recombinant expression system

A recombinant expression system was constructed, comprising pAphA, HapR and the Qrr4. These components are part of the quorum sensing mechanism in *V. cholerae*.

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In detail, the usability of pAphA as constitutive promoter for RFP expression was investigated and pAphA repressibility by HapR and its efficacy were elucidated. Differences in ability to repress pAphA by HapRs coming from two different serotypes of *V. cholerae* – the 01 and 037 strains – were found. These are due to a single point mutation in HapR from 037 strain (see Figure 5).

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To characterize the performance of the recombinant expression system, a single plasmid was generated, in which Red Fluorescent Protein (RFP) is expressed under control of the pAphA promoter. Via addition of arabinose the levels of HapR (SEQ ID NO 38) in the cells could be regulated. This means that by addition of different levels of arabinose into the medium the RFP fluorescence of the culture could be changed. The

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working principle of the system is shown in Figure 4.

In the next step, a double plasmid system, with the CqsS (SEQ ID NO 6), LuxU (SEQ ID NO 7) and LuxO (SEQ ID NO 8) genes expressed on one plasmid and GFP (SEQ ID NO 9) under pQrr4 promoter (SEQ ID NO 19) on another was generated. The working principle is shown in Figure 7. Thus, this system comprises the CAI-1 sensor module, in which phosphorylated LuxO activates the Qrr4 promoter. In the test construct the pBAD promoter drives LuxO, which is inducible by arabinose. Hence, only at high arabinose concentrations LuxO is expressed.

The results shown in Figures 8 and 9 demonstrate that the system was able to sense the presence of different concentrations of CAI-1 in the medium (the GFP fluorescence of the medium is a function of CAI-1 concentration). In other words, the system changes its fluorescence with different concentrations of CAI-1.

Example 2: Construction of a genetic CRISPR based genetic inverter

In this experiment it was determined, which part of the CRISPR system should be put under Qrr4 promoter control. To achieve this, the Cas9 protein (SEQ ID NO 39) was controlled by the ATc inducible promoter (SEQ ID NO 18) and the gRNA (SEQ ID NO 29) was put under control of an IPTG inducible promoter. The results of Figure 6 show that the dCas9 protein (SEQ ID NO 39) in tandem with the corresponding gRNA effectively represses expression from a promoter. The biggest advantage of this system is its versatility, as it can be used to represses nearly any desired promoter.

Example 3: Characterization of antimicrobial peptides

In this experiment the antimicrobial activities of Microcin S (SEQ ID NO 9), which is produced by probiotic *E. coli* G3/10 and synthetic peptides against *V. cholerae* and susceptible *E. coli* strains were investigated. Five synthetic peptides shown previously to have activity against *V. cholerae* and pathogenic *E. coli* were chosen. Two of the peptides G12.21 (SEQ ID NO 10) and G14.15 (SEQ ID NO 11) are granulysin based and were shown to effectively control the growth of *V. cholerae* both *in vitro* and *in vivo* (Da Silva et al. 2008). Similarly, two cationic peptides – CM15 (SEQ ID NO 13) and CM11 (SEQ ID NO 12) – which show inhibitory activity against *V. cholerae* and other pathogenic bacteria such as *P. aeruginosa*, *S. aureus*, *A. baumannii* and *E. coli* (Moghaddam et al. 2012) were tested. Moreover, the broad spectrum peptide, Magainin 2 (SEQ ID NO 14), which has a good killing efficiency on *V. cholerae* and pathogenic *E. coli* (Yaraksa et al. 2014) was chosen.

Moreover, for the secretion of Microcin S out of the producing strain, the YebF fusion protein (SEQ ID NO 15) was studied. YebF is a small, soluble endogenous protein, which can carry fusion proteins in their active states into the medium as early as 3h after induced expression.

5

To evaluate the activity of Microcin S (mcsS) and Microcin immunity protein (mcsI) in *E. coli* Top10 cells five different plasmids were constructed (Figure 10). Growth curves of *E. coli* harbouring different plasmids are shown in Figure 11. In order to directly demonstrate Microcin S activity against a susceptible *E. coli* strain (Top10), mcsS and mcsS-YebF were cloned separately into the pBbE8K vector resulting in the plasmids pBbE8K+McsS-YebF-I and pBbE8K+McsS-I (Table 1).

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Table 1: Sequences of the synthetic parts used for the expression of sensor proteins

BBa_B0032 (RBS)	SEQ ID NO 23
BBa_B0033 (RBS)	SEQ ID NO 24
BBa_B0034 (RBS)	SEQ ID NO 25
BBa_B0015 (Terminator)	SEQ ID NO 26
p66 (Promoter)	SEQ ID NO 22

15

Moreover, also the effect of Microcin immunity protein (mcsI) in the presence of Microcin S was studied. For this, the mcsI gene was introduced into the pBbE8K+McsS-YebF-I and pBbE8K+McsS-I plasmids resulting in pBbE8K+McsI+McsS-YebF-II and pBbE8K+McsI+McsS-II, respectively. In these constructs, mcsS expression is controlled by an araC pBAD activator-promoter (SEQ ID NO), rendering Microcin S expression inducible by L-arabinose. In contrast, mcsI expression is constitutively ON when using the rrnB promoter (SEQ ID NO 28).

20

When *E. coli* Top10 growing in liquid culture after being transformed with pBbE8K+McsS-YebF-I or pBbE8K+McsS-I were treated with 0.125% v/v L-arabinose the OD600 reading was significantly reduced compared to the un-induced and control cultures (p000k – vector devoid of any CDS) (Figure 11).

25

When *E. coli* Top10 cells growing in liquid culture after being transformed with pBbE8K+McsI+McsS-YebF-II or pBbE8K+McsI+McsS-II were treated with 0.125% v/v L-arabinose the OD600 reading was restored significantly compared to the un-induced and control cultures (p000k – vector devoid of any CDS) (Figure 11). This clearly

30

shows that mcsI immunity protein is acting as protease and the proteolysis of the Microcin S peptides restores growth in the host cell.

Therefore, the fusion of McsS to YebF does not hinder the Microcin S activity, as its killing potential is similar to that of McsS alone. Moreover, the McsI immunity protein supports the survival of the host cells, by protecting the host cells from the Microcin S activity, while the secreted Microcin S can target potential pathogens such as *V. cholerae*.

Furthermore, also the effect of Microcin whole cascade in *E. coli* Top10 growing in liquid culture with 0.125% arabinose and without arabinose after being transformed with construct 5 of Figure 12 was studied. It was found that *E. coli* expressing Microcin S had reduced OD600 readings after induction with arabinose (dark circles) compared to readings of the uninduced (unfilled inverted triangles) *E. coli* cells (Figure 12). This shows that Microcin S is effective in killing of *E. coli*.

In addition, the Microcin S peptide was purified to test its efficacy on *V. cholerae* and *E. coli*. For protein purification *E. coli* Top10 harbouring construct 1 of Figure 10 were grown in 250ml culture until 0.8 OD600 and Microcin S expression was fully induced by 0.125% arabinose for 2 hrs at 37°C. Subsequently, the cells were pelleted by centrifugation at 4000g for 20 min and lysed using a sonicator. Purification of Microcin S was carried out using Qiagen Ni-NTA fast start kit. The eluate was dialysed using Slide-A-Lyzer dialysis cassette and a 2 kDa cutoff (Life Technologies) for 4 hrs in 1X PBS. The dialysed samples were concentrated by ultra filtration using a molecular mass cutoff membrane (Amicon Ultra-15 Centrifugal Filter Unit, Millipore). As the estimated size of the protein is approximately 12 kDa, the first concentration was done using a 30 kDa cutoff membrane where the flow-through was collected and subsequently passed through a 3 kDa cutoff membrane. The resulting retentate was collected and the concentration was quantified using Quant-IT protein assay kit (Life Technologies).

The induced sample expressed has Microcin S (Figure 13B) and the fluorescence reading from the Quant-it assay was 1135, which was close to a concentration of 200 ng/μl, calculated from the standard curve (Fig 13A). No Microcin S was found in the uninduced sample as demonstrated by the SDS PAGE results (Figure 13B) and the fluorescence reading was 652. After the presence of Microcin S was confirmed from

the purified sample, a microbroth dilution assay was carried out to measure the minimum inhibitory concentration of purified Microcin S on *V. cholerae* and *E. coli*. 50 µl of each sample were serially diluted in 50 µl of LB broth in a 96 well plate. 50 µl of bacteria culture (*E. coli* wt/*V. cholerae*) were added to the diluted samples. The total volume was 100 µl with a runtime for 4hrs followed by a check of the OD and the killing efficiency. Minimal inhibition of both *V. cholerae* and *E. coli* was observed at 100 ng/µl of Microcin S (Figure 14). From these experiments it is clearly evident that purified Microcin S has inhibitory activity (Figure 15) against *V. cholerae* and *E. coli*.

Moreover, also the killing efficiency of the synthetic peptides listed in Table 2 below was studied.

Table 2: Synthetic peptides.

	Synthetic peptide
1	G12.21
2	G14.15
3	CM11
4	CM15
5	Magainin 2

For example, in the case of CM11 (P3) the P3 gene was cloned under the arabinose promoter (pBAD) in the pBbE8K vector resulting in the pBbE8K+P3 plasmid. In other words, P3 expression in this construct is controlled by an araC PBAD activator-promoter, rendering P3 expression inducible by L-arabinose. When, *E. coli* Top10 growing in liquid culture after being transformed with pBbE8K+P3, were treated with 0.2% v/v L-arabinose the OD600 reduced significantly compared to the un-induced sample (Figure 14). Thus, this directly demonstrates P3 activity against a susceptible *E. coli* strain.

Moreover, a minimum inhibitory concentration (MIC) assay was conducted using CM11 (P3). The medium used for the broth microdilution test was LB Broth and the cells were *V. cholera* A1522-Rugose-GFP and *E. coli* Nissile strains. Overnight cultures were transferred into 5 ml of LB and were grown at 37°C for 2-3 h. To approximate the density of 0.5 McFarland standard, this suspension with the inoculum concentration of 10⁸ colony forming units (CFU/ml) was then diluted to 10⁶ CFU/ml with the LB broth.

Peptide P3 stock solution of 1000 µg/ml was prepared using DMSO. Serial two-fold dilutions of the peptide (P3) with the following concentration (2 – 100 µg/ml) were prepared with LB broth and placed in 96-well microtiter plates and the lowest concentration inhibiting visible growth after 18-20 h at 37°C was recorded as MIC (see
5 Figures 16 and 17).

In addition, in order to characterize the lysis activity in *E. coli*, the lysis E7 gene was cloned under the tetracycline promoter (pTet) in the pBbA2C vector resulting in the pBbA2C+E7 plasmid. Hence, E7 expression is controlled by an pTet activator-
10 promoter, rendering E7 expression inducible by anhydrotetracycline (atc). When *E. coli* Top10 growing in liquid culture after being transformed with pBbA2C+E7, were treated with 200 nM anhydrotetracycline (atc) the OD600 reduced significantly due to the lysis compared to the un-induced sample (Figure 15).

Furthermore, gentamicin resistance was introduced into kanamycin resistant *E. coli* cells (E8K_aacC1) in order to be able to co-culture them with the target *V. cholera* cells. Our target strain has gentamicin resistance embedded into its chromosome. When tested with gentamicin and kanamycin *E. coli* Top10 harbouring E8K_aacC1 were able to grow normally, while the control strain *E. coli* (harbouring E8K_109),
20 which possesses only kanamycin resistance, was unable to grow in gentamicin (results not shown).

By "comprising" it is meant including, but not limited to, whatever follows the word "comprising". Thus, use of the term "comprising" indicates that the listed elements are
25 required or mandatory, but that other elements are optional and may or may not be present.

By "consisting of" is meant including, and limited to, whatever follows the phrase "consisting of". Thus, the phrase "consisting of" indicates that the listed elements are
30 required or mandatory, and that no other elements may be present.

By "about" in relation to a given numerical value, such as for temperature and period of time, it is meant to include numerical values within 10% of the specified value.

The inventions illustratively described herein may suitably be practiced in the absence
35 of any element or elements, limitation or limitations, not specifically disclosed herein.

Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the inventions embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

The invention has been described broadly and generically herein. Each of the narrower species and sub-generic groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

Sequence Listing

The sequences mentioned throughout the specification refer to the following:

	Name	Sequence	SEQ ID NO
1	pAphA	ggtcacaactttgtggcctttgtttacatttttcattattgagaataatgtcagttttcgact aatcagcatattgtattccactttatgcttattatttagatatactacgttccctctgtgataa gtaatgtaaagc	1
2	HapR	atggacgcatcaatcgaaaaacgcctcgaactcggctatcgctcaaaaacgcaa actacaactgatggaaatcgcggtggaagtgttgctaaacgcggcattggctgtggtg gtcacgcagatattgccgagattgcgcaagtcctcggtgcaacagtggtcaactactcc caactcgtgaagacttggtgacgatgtgctgaatttgggtcgtcagttactccaacttc ttgaccgatcacatcgatcttgattggatgtgaaaaccaacctacaaactctgtgcaa agagatgggtgaaattggcgatgaccgattgtcactggctcaaagtctggttgagtgga gtgctcaaccgtgacgaagttggccactgtttgttccaccaaccgaactaaccaa ctgctgatcagaaacatgtttatgaaagcgatggagcgtggcgaattgtgtgagaac acgatgtcgataacatggccagcctgttccacggcatcttctactccatcttctacaagt gaaccgttaggtgaacaagaagcagtgataagttggccgatagctacctaataatg ctgtgtatctataagaacta	2
3	CRISPR based genetic inverter gRNA	gacagctagcattgtaccaagtttagagctagaaaatagcaagttaaaataaggctag tccgttatcaactgaaaaagtggcaccgagtcgggtgcttttt	3
4	pQrr4	agttggcacgtaatctgcatttattagattg	4
5	dCas9	Atggataagaaatactcaataggcttagctatcggcacaaatagcgtcggtatgggcg gtgatcactgtgaatataaggttccgtctaaaaagtcaaggttctgggaaatacaga ccgccacagtatcaaaaaaatcttataggggtcttttattgacagtggagagaca gcggaagcgactcgtctcaaacggacagctcgtagaaggtatacacgtcggaaga atcgtattgttatctacaggagatttttcaaatgagatggcgaaagtagatgatagttc ttcatcgactgaagagctttttgtggaagaagacaagaagcatgaacgcatcct attttggaaatatagtagatgaagttgcttatcatgagaaatatccaactatctatcatct gcgaaaaaattggtagattctactgataaagcggatttgcgcttaatctatttggcctta gcgcatatgattaagttcgtgggtcatttttgattgaggagatttaaatcctgataatagt gatgtggacaaactatttccagttggtacaaacctacaatcaattattgaagaaaa ccctattaacgcaagtggagtagatgctaaagcgattcttctgcacgattgagtaaatc aagacgattagaaaaatctattgctcagctccccgggtgagaagaaaaatggcttattt gggaatctcattgcttgcattgggttgaccctaattttaaatcaaattttgattggcag aagatgctaaattacagcttcaaaagatacttacgatgatgatttagataatttattggc gcaaattggagatcaatatgctgattgttttggcagctaagaatttatcagatgctatttt actttcagatatcctaagagtaaataactgaaataactaaggctcccctatcagcttcaat	5

	gattaaacgctacgatgaacatcatcaagacttgactcttttaaagctttagttcgaca acaactccagaaaagtataaagaatcttttgatcaatcaaaaaacggatatgca gggtatattgatgggggagctagccaagaagaattttataaattatcaaccaatttta gaaaaaatggatgggtactgaggaattattgggtgaaactaaatcgtgaagattgctgc gcaagcaacggaccttgacaacggctctattccccatcaaattcacttgggtgagctg catgctatttgagaagacaagaagactttatccatttttaaagacaatcgtgagaag attgaaaaaatcttgacttttgaattccttattatgttggtccattggcgctggcaatagt cgtttgcattggatgactcggaagctgaagaaacaattaccccatggaattttgaaga agttgctgataaagggtgcttcagctcaatcattattgaacgcatgacaaacttgataa aaatcttccaaatgaaaaagtactaccaaacatagtttgctttatgagtattttacggtt ataacgaattgacaaagggtcaaatagtttactgaaggaatgcgaaaaccagcatttct ttcagggtgaacagaagaaagccattgttgatttactcttcaaaacaaatcgaaaagta accgttaagcaattaaaagaagattattcaaaaaatagaatgtttgatagtggtgaa atttcaggagttgaagatagatttaattgcttcattaggtacctaccatgatttgctaaaaat tattaaagataaagatttttggataatgaagaaaatgaagatatcttagaggatattgtt ttaacattgacctatttgaagatagggagatgattgaggaaagacttaaacatatgc tcaccttttgatgataaggtgatgaacagcttaaacgtcgccgttatactggtgggg acgttgtctcgaaaattgattaatggtattagggtataagcaatctggcaaaacaatatt agatttttgaaatcagatgggttgccaatcgcaattttatgcagctgatccatgatgata gtttgacatttaagaagacattcaaaaagcacaagtgtctggacaaggcgatagttt acatgaacatattgcaaatttagctggtagccctgctattaaaaaagggtattttacagac tgtaaaagtgttgatgaattgggtcaaagtaattggggcgccataagccagaaaatatc gttattgaaatggcacgtgaaaatcagacaactcaaaagggccagaaaaattcgcg agagcgtatgaacgaatcgaagaagggtatcaaagaattaggaagtcagattctta aagagcatcctgtgaaaatactcaattgcaaaatgaaaagctctatctctattatctcc aaaatggaagagacatgtatgtggaccaagaattagatattaatcgtttaagtattat gatgtcgatgccattgtccacaaagtttcttaaagacgattcaatagacaataaggctc ttaacgcgttctgataaaaatcggtgtaaatcggaataacgtccaagtgaagaagtagt caaaaagatgaaaaactattggagacaacttctaaacgccaagttaactactcaacg taagtttgataatttaacgaaagctgaacgtggagggttgagtgaacttgataaagctg gtttatcaaacgccaattggttgaaactcgccaaatcactaagcatgtggcacaattt tgatagtcgcatgaataactaaatacgtgaaaatgataaacttattcgagagggttaa agtgattaccttaaaaatctaaattagtttctgactccgaaaagattccaattctataag tacgtgagattaacaattaccatcatgccatgatgcgtatctaaatgccgtctgttgaa ctgcttgattaagaaatatccaaaactgaatcgagggttctatgggtgattataaagttt atgatgttcgtaaaatgattgctaagctgagcaagaaataggcaaagcaaccgcaa aatattcttttactctaataatcatgaacttctcaaaacagaaattacacttgcaaatgga gagattcgcaaacgcccctaatcgaaactaatggggaaactggagaaattgtctgg gataaagggcgagattttgccacagtcgcaaaagtattgtccatgccccaaagtcaata ttgtcaagaaaacagaagtacagacaggcggatttccaaggagtcattttaccaa	
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		aaagaaattcggacaagcttattgctcgtaaaaaagactgggatccaaaaaatatg gtggtttgatagccaacggtagcttattcagtcctagtggttgctaagggtgaaaaag ggaaatcgaagaagttaaaatccgttaaagagtactagggatcacaattatggaaa gaagttcctttgaaaaaatccgattgacttttagaagctaaaggatataaggaagtt aaaaaagacttaatacattaaactacctaataatagctcttttgagttagaaaaacggctgt aaacggatgctggctagtgccggagaattacaaaaaggaaatgagctggctctgcc aagcaaatatgtgaatttttatatttagctagtcattatgaaaagttgaagggtagtcca gaagataacgaacaaaaacaattgttggtggagcagcataagcattatttagatgag attattgagcaaatcagtgaattttctaagcgtgtatttttagcagatgccaattagataa agttcttagtgcataatacaaacatagagacaaaccaatacgtgaacaagcagaaa atattattcattatttacgttgacgaatcttgagctcccgtgcttttaaatattttgataca acaattgatcgtaaacgatatacgtctacaaaagaagtttttagatgccactcttatccat caatccatcactgggtctttatgaaacacgcattgattgagtcagctaggaggtgactaa	
6	CqsS	MIVSMDVIKRVYQYAEPNLSLVGWMGMLGFPAYYFIWEYWF PQSYENLGLRCAAVALFGGLVFRDSMPKKWQRYMPGYFLF TIGFCLPFFFAFMMLMNDWSTIWAMSFMASIFLHILLVHDTRV MALQALFSVLVAYLAVYGLTDFHPTTLIEWQYIPIFLFTYVFGN LCFFRNQISHETKVSIAKTFGAGIAHEMRNPLSALKTSIDVVR TMIPKPQTAHTDYSLDAQELDLLHQILNEADDVIYSGNNAID LLTSIDENRVSPASFKKHSVVDVIEKAVKTFPYKNAADQHSV ELEVHQPFDFFGSDTLLTYALFNLLKNAFYQKEHFSVCISIE QTSEHNLIRVRDNGVGIAPEMLEDIFRDFYTFGKNGSYGLGL PFCRKVMSAFGGTIRCASQQGQWTEFVLSFPRYDSDTVNEI KTELLKTKSLIYIGSNQAIVRELNQLAVEDEFGFTAISAQQAVR RQDYEFEFDLILLDLDDATAQGELLPKLEGTL SFAEGCIGYVY DPGKTYAVNINRYLRIQPISIHSLRKPRKIIERLLFEQESLMN RNVIPLQKSRHERRILVVDNQSIRTFTAILLEQQGYEVVQAN DGSEVLKHMESQNIDLVLMDIEMPVNGGLEATRLIRNSEHEY KNIPIGYTGDNSPKTLALVQTSGMNDFIVKPADRDVLLNKVA AWV	6
7	LuxU	MREWINSKIDLLAKEIGEENVPILVNIFLGELNDYQSKLVSDT VADKLGYLKEISHALKSSAASFGADRLCAKAVELDSRAKSGE MMDISLEVEHMLELLKQTHQCYSIDL VH	7
8	LuxO	MVEDTASVAALYRSYLTPLDIDINIVGTGRDAIESIGRREPDLI LLDLRLPDMTGMDVLYAVKEKSPDVPIVFMATAHGSIDTAVEA MRHGAQDFLIKPEADRLRVTVNNAIRKASKLKNDVDNKNQ NYQGGFIGSSQTMQAVYRTIDSAASSKASIFITGESGTGKEVC AEAIHAASKRGDKPFIAINCAAIPKDLIESELFHVKGAFTGAA TERQGAAEAADGGTLFLDELCEMDLDLQTKLLRFIQTGTGTFQK VGSSKMKSVDRFVCATNRDPWKEVQEGRFREDLYYRLYVI	8

		PLHLPPLRARGDDVIEIAYSLLGFMSKEEGKDFVRLSAEVVE RFRQYEWPGNVRQLQNVLRNVVVLNEGRETLDMLPPPLNQ MSAPINRALPLAHENKVSVEIFPLWMTEKQAIEQAIEACDG NIPRAATYLDVSPSTIYRKLQTWNEKVKEKEKER	
9	Microcin peptide	S MSNIRELSFDEIALVSGGNANSNYEGGSRSRNTGARNSLG RNAPTHIYSDPSTVKCANAVFSGMVGGAIKGGPVGMTGTI GGAVIGQCLSGGGNGNGGGNRAGSSNCSGNSVGGTCSR	9
10	G12.21	rrvsrrpmrrysrrprlv	10
11	G14.15	grsrwrrvsrrfmrr	11
12	CM11	WKLFFKILKVL-NH2	12
13	CM15	KWKLFFKIGAVLKVL-NH2	13
14	Magainin 2	GIGKFLHSAKKFGKAFVGEIMNS	14
15	YebF	MKKRGAFGLLLLVSACASVFAANNETSKSVTFPKCEDLDAA GIAASVKRDYQQNRVARWADDQKIVGQADPVAWVSLQDIQ GKDDKWSVPLTVRGKSADIIHYQVSDCKAGMAEYQRR	15
16	McsS-YebF	MSNIRELSFDEIALVSGGNANSNYEGGSRSRNTGARNSLG RNAPTHIYSDPSTVKCANAVFSGMVGGAIKGGPVGMTGTI GGAVIGQCLSGGGNGNGGGNRAGSSNCSGNSVGGTCSKK RGAFGLLLLVSACASVFAANNETSKSVTFPKCEDLDAAGIAA SVKRDYQQNRVARWADDQKIVGQADPVAWVSLQDIQGKDD KWSVPLTVRGKSADIIHYQVSDCKAGMAEYQRR	16
17	IPTG inducible promoter	aattgtgagcggataacaattgacattgtgagcggataacaagatactgagcac	17
18	ATc inducible promoter	cctaattttgtgacactctatcggtgatagagtattttaccactccctatcagtgatagag aaaa	18
19	GFP	MRKGEELFTGVVPIVLVDGDVNGHKFSVSGEGEGDATYGK LTLKFICTTGKLPVPWPTLVTTFGYGVQCFARYPDHMKQHD FFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIE LKGIDFKEDGNILGHKLEYNNSHNVYIMADKQKNGIKVNFKI RHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHVLTQSALSK DPNEKRDHMLLEFVTAAGITHGMDELYK	19
20	RFP	MASEDVIKEFMRFKVRMEGSVNGHEFEIEGEGEGRPYEGT QTAKLKVTKGGPLPFAWDILSPQFQYGSKAYVKHPADIPDYL KLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKL RGTNFSPDGPVMQKKTMGWEASTERMYPEDGALKGEIKMR LKLKDGGHYDAEVKTTYMAKKPVQLPGAYKTDIKLDITSHNE DYTIVEQYERAEGRHSTGA	20
21	pBAD	ctgacgcttttatcgcaactctctactgt	21
22	p66	gtatatattaaaacattcttgacatcttgaaacaaatatgatataatagcaatatat	22

23	Bba_B0032	tctagagtcacacaggaaagtactag	23
24	Bba_B0033	tctagagtcacacaggactactag	24
25	Bba_B0034	aaagaggagaaa	25
26	BBa_B0015	aggatctccaggcatcaaataaaacgaaaggctcagtcgaaagactgggcctttcgt ttatctgtgtttgtcgggtgaacgctctctactagagtcacactggctcaccttcgggtgg gcctttctgcgtttata	26
27	araC pBAD promoter	ttatgacaacttgacggctacatcattcactttttttcacaaccggcacggaactcgctc gggctggcccggtgcatttttaataaccgcgagaaatagagttgatcgtaaaac caacattgcgaccgacgggtggcgataggcatccgggtgggtgctcaaaagcagcttc gcctggctgatacgttggctcgcgccagcttaagacgctaataccctaactgctggcg gaaaagatgtgacagacgacggcgacaagcaaacaatgctgtgcgacgctggc gatatcaaaattgctgtctgccagggtgatcgctgatgtactgacaagcctcgcgtacc gattatccatcggtggatggagcgactcgtaatacgttccatgcgccgcagtaacaat tgctcaagcagatttatcgccagcagctccgaatagcgccctccccttggccggcgtt aatgatttgcccaaacaggctcgctgaaatgcggtggtgcgcttcacggggcgaaa gaaccccgatttggcaaatattgacggccagtttaagccattcatgccagtaggcg cggacgaaagtaaaccactgggtgataccattcgcgagcctccggatgacgacgt agtgtgaatctctcctggcggaacagcaaaatacaccgggtcggaacaaatt ctcgtccctgattttcaccacccctgaccggaatggtgagattgagaatataacctt cattcccagcgggtcggtcgataaaaaaatcgagataaccgttggcctcaatcggcgtt aaacccgccaccagatgggcattaaacgagtatcccggcagcaggggatcatttg cgcttcagccat	27
28	rrsBP1 promoter	ttgtcaggccggaataactccctataat	28
29	Genetic inverter sRNA (Qrr4)	gacccttctaagccgaggggtcacctagccaactgacgttgtagtgaaacaccattgttc acacttatagacggccaatcacacttctgtggtggccttttttt	29
30	L1	NGVQPKY	30
31	L2	NAGSLLSGWG	31
32	B1	LIAGLAANFLPKLFCKITK	32
33	CP1	SWLSKTAKKLENSAKKRISIEGIAIAIQGGPR	33
34	FlgM	ATGAGTATTGATCGCACTTCGCCTCTGAAGCCTGTAAGCA CCGTTCAACCGCGCGAAACCACTGACGCGCCGGTAACGA ACAGCCGGGCGGCAAAAACAACCGCCTCCACCAGCACCA GTGTGACGTTAAGCGACGCGCAAGCAAACTGATGCAAC CCGGCAGCAGTGATATCAATCTTGAACGTGTGAAGCGTT AAAACCTGGCGATTTCGTAACGGTGAACATAAAATGGACACC GGCAAAATTGCCGATGCGCTGATCAACGAAGCGCAGCAA GACTTGCAGAGTAAC	34

35	Lysis E7	atgaaaaaaataacagggattattttattgcttctgcagccattattctgctgcatgtca ggcaaaactatatccgtgatgttcagggcgggacagtatcaccgtcgtcaactgctgaa ctgaccggagtggaaacgcagtaa	35
36	aacC1	ATGTTACGCAGCAGCAACGATGTTACGCAGCAGGGCAGT CGCCCTAAAACAAAGTTAGGTGGCTCAAGTATGGGCATCA TTCGCACATGTAGGCTCGGCCCTGACCAAGTCAAATCCAT GAGGGCTGCTCTTGATCTTTTCGGTCGTGAGTTCGGAGAC GTAGCCACCTACTCCCAACATCAGCCGGACTCCGATTACC TCGGGAACTTGCTCCGTAGTAAGACATTCATCGCGCTTGC TGCCTTCGACCAAGAAGCGGTTGTTGGCGCTCTCGCGGC TTACGTTCTGCCAAAGTTTGAGCAGGCGCGTAGTGAGATC TATATCTATGATCTCGCAGTCTCCGGCGAGCACCGGAGG CAAG3GCATTGCCACCGCGCTCATCAATCTCCTCAAGCAT GAGGCCAACGCGCTTGGTGCTTATGTGATCTACGTGCAA GCAGATTACGGTGACGATCCCGCAGTGGCTCTCTATACAA AGTTGGGCATACGGGAAGAAGTGATGCACTTTGATATCGA CCCAAGTACCGCCACCTAA	36
37	CM11 (P3)	tggaaactgTTTAAAAAATTCTGAAAGTGCTGAACCAT	37
38	HapR Protein	MDASIEKRPRTLSPQKRKLQLEIALEVFAKRGIGRGGHAD IAEIAQVSVATVFNYFPTREDLVDDVLNFVVRQYSNFLTDHID LDLDVKTNLQTLCKEMVKLAMTDCHWLKVWFEWSASTRDE VWPLFVSTNRTNQLLIRNMFMKAMERGELCEKHDVDNMSL FHGIFYSIFLQVNRLEGEQEA VYKLADSYLNMLCIYKN	38
39	dCas9 protein	MDKKYSIGLAIGTNSVGWAVITDEYKVP SKKFKVLGNTDRHSI KKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIF SNEMAKVDDSFHRL EESFLVEEDKKHERHPIFGNIVDEVAY HEKYPTIYHLRKKLV DSTDKADLR LIYLALAHMIKFRGHFLIEG DLNPDNSDVKLFIQLVQTYNQLFEENPINASGVDAKAILSAR LSKSRRL ENLIAQLPGEKKNGLFGNLIALSLGLTPNFKS NFDL AEDAKLQLSKD TYDDLDNLLAQIGDQYADLFLAAKNLSDAIL LSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLP EKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTE ELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFY PFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETI TPWNFEVVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSL YEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTN RKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLL KIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLF DDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKS DGFANRNF MQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANL AGSPA IKKGILQTVKVVDELVKVMGRHKPENIVIAMARENQTT QKGQKNSRERMKRIEEGKELGSQILKEHPVENTQLQNEKLY LYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDN KVLTRSDKNRGKSDNVPSEEVVKMKMKNYWRQLLNAKLITQR KFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSR MNTKYDENDKLIREVKVITLKS KLVSDFRKDFQFYKVREINNY HHAHDAYLNAVVG TALIKKYPKLESEFVYGDYKVYDVRKMIA KSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNG ETGEIVWDKGRDFATVRKVL SMPQVNIVKKTEVQTGGFSKE	39

		SILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVAKVE KGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKD LIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFL YLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKR VILADANLDKVLSAYNKHDKPIREQAENIIHLFTLTNLGAPAA FKYFDTTIDRKRYTSTKEVLDTLIHQSI TGLYETRIDLSQLGG D	
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Claims

1. A recombinant expression system comprising at least:
 - (i) a first nucleotide sequence encoding for at least one protein of a quorum sensing system capable of detecting the presence, amount or both of a microorganism of interest by forming a complex with a marker molecule indicating the presence of said microorganism;
 - (ii) a second nucleotide sequence encoding for at least one antimicrobial peptide, wherein the at least one antimicrobial peptide is effective against the microorganism of interest detected by the at least one protein encoded by the first nucleotide sequence,
 - (iii) a third nucleotide sequence encoding for a genetic inverter that inhibits expression of the second nucleotide sequence,wherein the genetic inverter is under control of an inducible promoter and wherein the inducible promoter is induced, if the complex of the at least one protein encoded by the first nucleotide sequence and the marker molecule indicating the presence of said microorganism is below a threshold concentration and is not induced, if the complex of the at least one protein encoded by the first nucleotide sequence and the marker molecule indicating the presence of said microorganism exceeds a threshold concentration.
2. The recombinant expression system according to claim 1, wherein the genetic inverter (a) comprises a nucleotide sequence encoding a repressor molecule and a promoter repressed by the repressor, with said promoter controlling expression of the second nucleotide sequence, (b) comprises a nucleotide sequence encoding an sRNA specific for the mRNA encoded by the second nucleotide sequence or (c) is a CRISPRi system comprising a nucleotide sequence encoding for a gRNA sequence specific for the second nucleotide sequence and a nucleotide sequence encoding for dCas9 protein.
3. The recombinant expression system according to claim 1 or 2, wherein the genetic inverter is a CRISPRi system, wherein the nucleotide sequence encoding for a gRNA sequence specific for the second nucleotide sequence is under control of the inducible promoter and the nucleotide sequence encoding for dCas9 is constitutively expressed.

4. The recombinant expression system according claims 1-3, wherein the inducible promoter controlling the genetic inverter is the Qrr4 promoter (SEQ ID NO 4).
5. The recombinant expression system according to claims 1-4, wherein the detection mechanism of the first nucleotide sequence is based on the CqsS/CAI-1 quorum-sensing phosphorelay system of *V. cholerae*.
6. The recombinant expression system according to claims 1-5, wherein the first nucleotide sequence encodes for the proteins of the (S)-3-hydroxytridecan-4-one (CAI-1) sensor module and thus comprises the nucleotide sequence encoding for the LuxO protein having the amino acid sequence as set forth in SEQ ID NO: 8, the nucleotide sequence encoding for the CqsS protein having the amino acid sequence as set forth in SEQ ID NO: 6 and the nucleotide sequence encoding for the LuxU protein having the amino acid sequence as set forth in SEQ ID NO: 7.
7. The recombinant expression system according to claims 1-6, wherein the one or more antimicrobial peptide(s) is selected from the group consisting of Microcin S (McsS, SEQ ID NO 9), CM11 (SEQ ID NO 12), CM15 (SEQ ID NO 13) and Magainin 2 (SEQ ID NO 14).
8. The recombinant expression system according to claims 1-7, wherein the antimicrobial peptide is fused to a secretory peptide.
9. The recombinant expression system according to claims 1-8, wherein the antimicrobial peptide is fused to the YebF (SEQ ID NO 15) secretory peptide.
10. The recombinant expression system according to claim 9, wherein the antimicrobial peptide McsS (SEQ ID NO 9) is fused to the YebF peptide (McsS-YebF, SEQ ID NO 16).
11. A recombinant cell comprising a recombinant expression system according to claims 1-10.
12. The recombinant cell of claim 11, wherein the recombinant cell is a genetically engineered prokaryotic cell.

13. The recombinant cell of claim 12, wherein the prokaryotic cell is a probiotic bacterium.

14. The recombinant cell of claim 13, wherein the prokaryotic cell is an *Escherichia coli* cell.

15. The recombinant cell of claim 14, wherein the *Escherichia coli* cell is a probiotic *E. coli* Nissle cell.

16. A method of sensing and killing pathogenic microorganisms, the method comprising contacting the recombinant cell of any one of claims 11-15 with the microorganism of interest.

17. The method of claim 16, wherein the microorganism of interest is a human pathogen.

18. The method of claim 17, wherein the pathogenic microorganism is selected from the group consisting of *Pseudomonas aeruginosa*, *Clostridium difficile*, *Escherichia coli*, *Helicobacter pylori*, *Salmonella*, *Vibrio cholerae* and *Yersinia*.

19. Use of a recombinant expression system comprising at least:

(i) a first nucleotide sequence encoding for at least one protein of a quorum sensing system capable of detecting the presence, amount or both of a microorganism of interest by forming a complex with a marker molecule indicating the presence of said microorganism;

(ii) a second nucleotide sequence encoding for at least one detectable marker,

(iii) a third nucleotide sequence encoding for a genetic inverter that inhibits expression of the second nucleotide sequence,

wherein the genetic inverter is under control of an inducible promoter and

wherein the inducible promoter is induced if the complex of the at least one protein encoded by the first nucleotide sequence and the marker molecule indicating the presence of said microorganism is below a threshold concentration and is not induced if the complex of the at least one protein encoded by the first nucleotide sequence and the marker molecule indicating the presence of said microorganism exceeds a threshold concentration,

for detection of at least one type of microorganism.