BMC-069

Identification and biosynthesis of an autoinducing cyclopeptide from *Clostridium acetobutylicum* that regulates solvent biosynthesis and cell morphology

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Autoinducing peptides (AIPs) are a type of signaling molecule that is secreted and sensed by quorum sensing (QS) systems to modulate population behavior and cellular physiology. However, the minute amounts make it difficult to detect, and their synthetic pathway remains elusive, leading to a very limited knowledge of the essence of AIPs as well as their functions. Here we have identified the native structure and achieved the direct quantification of an AIP signal in clostridia, and further characterized the proteases involved in AIP synthesis. The method described here enables rapid and accurate quantitation of AIP contained in a few microliters of culture supernatant. Except for type I signal peptidase which is generally considered involved in the N-terminal processing of intermediate, we identified three homologs of CAAX metalloprotease related to the second step of AgrD processing in C. acetobutylicum via bioinformatics and mutational analysis. Furthermore, the AIP displayed sophisticated biological functions in modulating physiological processes, extending the limited cognition of signaling molecules in clostridia. AIP formation was completely abolished via in-frame deletion of agrD, which led to a 42.3% increase in biomass. The agrBD overexpression strain showed intensified AIP production of 4.4-fold enhancement and improved solvent production by 18.7%, maintaining a cell morphology with high metabolic activity. This study provided a novel strategy for bioprocess performance improvement by manipulating cell-cell communication, such as the development of a cheap and easily available QS agonist, and the discovery and determination of AIP facilitate further studies of potential effects in microbiota.

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BMC-070

Metal adsorption improvement of *Saccharomyces cerevisiae* by yeast surface display

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High exposure to heavy metals (HMs) is extremely toxic to living organisms, and environmental decontamination of HMs is mainly done by physicochemical processes, with additional ecological impact.

Microbial bioremediation has been proposed as a suitable ecological alternative to remove HMs due to the adsorption capacity of microorganisms that are capable of reversibly sequestering the ions. In particular, S.cerevisiae has been proposed for the possibility of increasing its adsorptive capacity through genetic modifications that allow the expression of particular proteins on the yeast surface.

Here, for the first time, we modify natural yeast strains to arm their cell surface by expressing metal-binding proteins capable of selectively binding metal ions (Cu2+, Ni2+). We characterize the binding properties of the strains by performing various functional and biochemical assays.

Overall, our data demonstrate that natural strains are capable of expressing surface-localized proteins, further supporting the significant improvement of armed yeast cells in capturing and recovering metal ions from contaminated solutions, making them suitable for bioremediation. The technological perspectives of using our modified strains are also discussed.

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BMC-071

Development of a dual biocatalyzed anode and cathode microbial electrosynthesis system for simultaneous carbon dioxide sequestration and glycerol biodegradation

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The on-going climate crisis and accelerating production of greenhouse gases such as carbon dioxide has sparked a sense of urgency in the scientific community to come up with solutions to sequestrate and/or recycle CO2. Microbial electrosynthesis (MES) has emerged as a renewable and green technology that uses electroactive microbial catalysts to mineralize and treat different wastes/pollutants such as CO2. This study aims to develop a novel dual biocatalyzed MES consisting of an efficient gas diffusion biocathode for CO2 sequestration in combination with a bioanode for simultaneous glycerol valorization. The use of biocatalysts both at the anode and cathode in a dual biocatalytic MES can simultaneously treat CO2 and glycerol along with bio production of chemicals making the process more energy and cost efficient. The developed system will minimize the carbon footprint mitigating climate change with a significant scientific and societal impact. The decreasing CO2 emissions and simultaneous biotransformation of wastes in the bioanode will have a great societal impact resolving not only the long going carbon capture crisis but at the same time presenting a sustainable platform for environmental remediation and chemical production. The use of dual bio-catalysed MES will create new knowledge in biosynthesis, power and fuel generation along with persistent pollutant treatment with bioelectrochemical systems using microbes.

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Frontiers of Biotechnology I
FBI-001

Transcription factor-based biosensors for application in autotrophic bacteria

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Inducible gene expression systems, composed of transcription factor (TF) and cognate inducible promoter have come into the focus as a platform for the development of genetically encoded TF-based biosensors. The interaction of TFs with specific inducers activates the expression of the reporter protein in a dose-dependent manner, resulting in quantitatively measurable output. TF-based biosensors have been employed as synthetic biology devices and enable the development of microbial cell factories using high-throughput screening strategies. Native TFs and promoter pairs are widespread and are adapted to the organism's purposes, for this reason, TF-based biosensors are often limited in sensitivity and are incompatible with non-native hosts. Although several TF-based biosensors were applied in different α-, β-, and γ-proteobacteria, however, detection of inducers with biosensors in non-native host cells remains a challenge and their use in purple phototrophic bacteria is limited so far.

Previously, we developed and characterized TF-based biosensors for organic and phenolic acids. Several inducible gene expression systems induced by phenolic acids, including o-hydroxybenzoic acid, m-hydroxybenzoic acid, vanillic acid, and protocatechuic acid, have been predicted to be applicable in well-studied microorganisms E. coli, C. necator, and P. putida. Additionally, we utilize a protocatechuic acid-biosensor in E. coli to identify enzymes with enhanced activity for the conversion of p-hydroxybenzoate to protocatechuate. These systems are currently being developed for the purple photoautotrophic bacterium R. capsulatus.

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FBI-002

Nucleic acid-based biosensors: advancing biomolecule detection for rapid phenotyping applications

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Accurately quantifying biomolecules in complex biological matrices is crucial for advancements in medicine, biotechnology, and agriculture. However, current biomolecule detection technologies, such as chromatography-mass spectrometry and immunoassays, often require specialized laboratories and trained operators, which limit their availability, processing capacity, and utility, especially in urgent scenarios.

Protein-based biosensors have shown promise in research and industry applications in recent years. This study explores the potential of nucleic acid-based binding entities, such as DNA aptamers, as biosensors for high-value therapeutic targets like toxic drugs and disease biomarkers.

Our project focuses on building synthetic sensing devices based on the recognition capability of nucleic acids. These biosensors leverage the diverse conformational space, exceptional stability, and structural flexibility of DNA aptamers. Initially, we use this technology to develop biosensors for small molecules, such as methotrexate (MTX), an immune system suppressant that requires close patient monitoring. Subsequently, we applied this methodology to establish sensors for heart failure biomarkers in saliva, including Human S100A7 and Galactin-3.

Using SELEX technology, we isolated specific DNA binders to S100A7, Gal3, and MTX, which we used to create fluorescent and colorimetric tests. Our findings underscore the potential of integrating aptamers into point-of-care devices for challenging molecular targets.

Beyond its scientific significance, this project showcases the development of synthetic sensing devices for enhanced biomolecule detection in diverse applications, such as analyzing bioproducts, pharmaceuticals, disease biomarkers, and toxins. By unlocking the potential of nucleic acid-based biosensors, we aim to pave the way for rapid and accessible biomolecule quantification at the point of need.

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FBI-006

A filter based whole-cell sensor design for measuring in bioreactors

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Microorganisms constitute invaluable tools in various fields of industrial biotechnology. One of these applications is the bioengineering of microbes to serve as whole-cell sensors capable of sensing analytes present within their surroundings and generating signals which can be easily measured using optical and/or electrical techniques. Here, we use commercial filter membranes and Escherichia coli bacteria to develop whole-cell sensors for bioreactor monitoring. Specifically, we employ an E. coli K-12 BW25113 strain engineered to constitutively express the gene for superfolder green fluorescent protein (sfGFP) under the PJ23106 promoter in a pSEVAb23 vector, resulting in in vivo production of green fluorescence. The fluorescence intensity generated by the bacteria is measured using an optical sensor consisting of a chamber which holds a polyethylene terephthalate (PET) membrane and optical components created via three-dimensional (3D) printing. The PET porous film confines the engineered E. coli within the chamber preventing their escape into the surrounding medium while allowing nutrient diffusion. The optical readout is established by a light emitting diode (LED) excitation light source guided by a light pipe towards the microorganisms. Another light pipe collects and directs the emitted fluorescent light towards a photodetector. Optical filters are used to select the right wavelength ranges for the blue excitation and green emitted light. Initial promising results with our manufactured prototype demonstrate the feasibility of this sensor design, which has broader potential beyond bioreactor cultures and can be exploited in other (bio)sensing applications.

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