Colorimetric Whole-Cell Biosensors

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Colorimetric whole-cell biosensors are natural or genetically engineered microorganisms utilized to detect target molecules and ions as indicators of pollutants and biological activity in the environment. Upon detection, within specific concentration ranges which vary depending on the microorganism and its genetic circuitry among other factors, these sensors produce pigments which can be detected with the human eye past certain thresholds and quantified using simple analytical techniques, namely spectrophotometry. These sensors, which can be rendered portable through lyophilization and other methods, provide valuable and reliable substitutes of more demanding analytical ex situ techniques.

Keywords: whole-cell biosensors ; pigments ; quantification ; qualification ; spectrophotometry

1. Introduction

A biosensor is a measurement or quantification system relying on a biological component to recognize target analytes [1]. These devices quantify biological activity or chemical composition through the production of a dose-dependent signal [2]. Whole-cell biosensors (WCBs) are genetically engineered microorganisms capable of detecting and reporting a particular compound or analyte through the emission of a discernable signal using a stimulus-specific reporter system. WCBs are efficient and cost-effective means for obtaining in situ qualitative as well as quantitative information about the medium in which they are introduced. The precision of the readout is governed by a number of factors including the microbial chassis used as a sensor, its resistance to the concentration of the substance being quantified, and the metabolic burden incurred by the synthesis of the output molecule among others. While some biosensors are capable of producing a single readout in response to a specific analyte, others have been engineered to produce distinct concentration-specific outputs [3]. Broadly speaking, these systems utilize a sensing module which detects a specific target (e.g., ion, molecule, or metabolite) and transmits this stimulus to a reporting module which outputs a visible signal. A panoply of characterized biological sensing systems and signaling pathways could be implemented in WCB sensing mechanism design [4]. Transcriptional regulator systems integrate promoters responding to specific environmental constituents linked to engineered gene circuits [5], resulting in the expression or repression of the reporter genes when the promoter-specific compound or protein-ion complex is detected in the medium. Another system relies on a riboswitch comprising an RNA aptamer. Through a conformational change induced by specific metabolite or ligand-binding, the riboswitch may regulate the expression of reporter genes through different mechanisms: halting reporter transcription through the inhibition of antiterminator or the cleavage of mRNA, or activating or repressing translation via the sequestration of the ribosomal binding site (RBS) [6][7]. In essence, WCBs exploit the sensitivity of natural regulatory systems crucial to the survival of microorganisms [8]. They can be utilized for multifarious purposes, such as monitoring natural environments like soil or bodies of water [9][10], screening for high-output strains in biosynthetic industrial settings [11], or providing health data by revealing the amounts of specific micronutrients in human serum among other uses [12].

2. Response of WCBs to Synthetic Molecules

Polychlorinated biphenyls (PCBs) designate a large category of synthetic organic molecules with high hydrophobicity. The potential health complications engendered by PCBs are multitudinous and include neurological conditions, endocrine disruptions, and cancer [13]. Before their worldwide ban through the Stockholm Convention on Persistent Organic Pollutants in 2001, which superseded their 1976 ban in the USA, PCBs were used in a broad range of applications including textiles, construction, transformer oils, and hydraulic equipment. Despite the interruption of their production, the noxious effects of PCBs persist given their capacity to cause complications at remarkably low concentrations. To that end, PCB sensors with sensitivities in the ppb domain had been devised [14][15].

The sensor developed by Gavlasova et al. harnesses the metabolic abilities of *Pseudomonas* sp. P2 [16]. This strain isolated from a PCB contaminated soil, can, in the presence of biphenyl, convert PCBs into chlorobenzoic acid following a four-step catabolic pathway (**Figure 1**) [17][18].

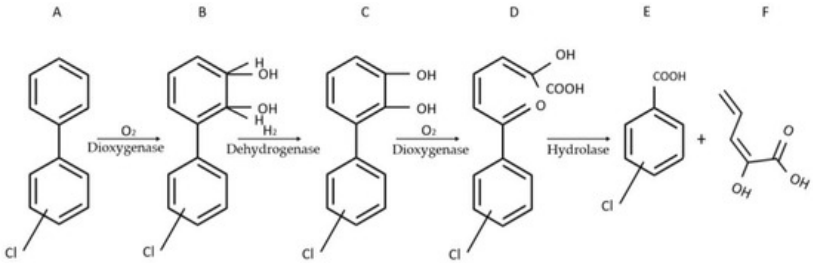

Figure 1. Four-step catabolic pathway of PCBs in *Pseudomonas* sp. P2. Adapted from Gavlasova et al. [16]. (**A**) biphenyl; (**B**) 2,3-dihydro-2,3-dihydroxybiphenyl; (**C**) 2,3-dihydroxybiphenyl; (**D**) 2-hydroxy-6-oxo-6-phenyl-2,4-hexadienoic acid (HOPDA); (**E**) benzoic acid; (**F**) 2-hydroxy-2,4-pentadienoic acid.

In its penultimate step, the pathway engenders the formation of readily observable yellow HOPDA. Through its maximum absorbance at λ = 398 nm, HOPDA produced by *Pseudomonas* sp. P2 can qualitatively signal the presence of PCBs and semi-quantitatively determine their amounts. While a protocol leveraging HOPDA synthesis had been previously devised by Kuncova et al. [19], suboptimal immobilization of the bacterial cells on glass beads resulted in inaccurate quantification. The innovative aspect of the protocol developed by Gavlasova and colleagues lies in its use of tetramethylorthosilicate (TMOS) to immobilize the homogeneously dispersed WCB on 3 cm petri dishes.

With its myriad chemical compositions and physical properties, soil nurtures numerous interdependent systems of flora and fauna influenced by its attributes and which, in turn, influence its composition. As such, deleterious alterations made to any component of this system can inevitably yield commensurate ripple effects with enduring consequences. Human agricultural activity has entailed the use of a number of pesticides with considerable impact on animals and human health [20]. A number of optical biosensors have been devised to detect organophosphate pesticides through their hydrolysis products, chief among which is 4-nitrophenol [21].

To detect the hydrolysis products of organophosphate pesticides, Chong and Ching successfully produced an *E. coli* colorimetric WCB using a modified DmpR transcriptional activator, which allowed greater effector specificity and thus higher expression level of its cognate promoter driving the expression of the monomeric red fluorescent protein 1 (*mRFP*) reporter gene [22]. While lycopene would be an obvious choice should a red pigment be considered as the output signal, the researchers stipulate that the synthesis of lycopene and carotenoids in general is highly influenced by metabolic fluxes. These variabilities might result in inconsistent results from a color intensity perspective and an incubation time standpoint. The use of readily visible red fluorescent proteins, expressed through *mRFP1*, was deemed likely to engender more dependable results [22]. Transcription regulator DmpR, widely investigated in phenol detection contexts [23][24][25], was used as a sensing module in a wholly mutagenized form. To select 4-nitrophenol sensing mutants most conducive to discernable red fluorescent protein (RFP) synthesis, the researchers used DmpR as a sensing module and *mRFP1* as a reporter module. *DmpR* was mutagenized randomly before reconstituting the sensor plasmid and transforming it into *E. coli* MG1655. The transformed *E. coli* strains were cultured on LB agar plates and, after accounting for possible false positives, two 4-nitrophenol-effected *DmpR* mutants DM01 and DM12 with adequate pigment basal expression were retained. Each of the transcription regulators was cloned onto a plasmid bearing *oph*, a gene encoding organophosphorus hydrolase or OPH, and *mRFP1* to create novel sensor plasmids. Organophosphorus hydrolase enables the hydrolysis of parathion into 4-nitrophenol and thus its detection through DM01 and DM12, followed by the induced synthesis of RFPs. The novel biosensors were able to signal the presence of both hydrolyzed and unhydrolyzed organophosphorus pesticides. The synthesis of organophosphorus hydrolase (OPH) commensurably affected the effectiveness of the sensor and, to overcome this bottleneck, a strong constitutive promoter *pTet* was selected to drive the expression of the *oph* gene in the engineered DM01 and DM12 mutant *E. coli* strains.

3. Detection of Metals by WCBs

3.1. Response of WCBs to Copper

Copper (Cu), among other metal ions such as zinc and manganese, plays a significant role as an enzyme cofactor involved in the catalysis of metabolic activity and the maintenance of cell integrity through osmotic pressure regulation [26]. Despite its considerable utility, copper becomes harmful to humans, animals, and plants alike past respective thresholds, and colorimetric WCBs can provide reasonable data regarding its concentration in water bodies. Several copper metalloregulator systems have been characterized in a number of microorganisms. In *Saccharomyces cerevisiae* for example, metallothionein encoded in *CUP1* enables the chelation of Cu(II) ions and protects the cell from copper

poisoning. The copper-dependent DNA-binding protein ACE1 induces *CUP1* transcription through the binding of ACE1-Cu(II) complex onto the upstream activation sequence of *CUP1* [27].

A colorimetric WCB consisted of an engineered strain of *S. cerevisiae* with a deleted *ADE2* gene, encoding phosphoribosylaminoimidazole carboxylase, and a *CUP1* promoter *PCUP1* driving the expression of genes at the *ADE5,7* locus encoding glycinamide ribotide synthetase and aminoimidazole ribotide synthetase [28][29]. In this strain dubbed BY-*ade2*-P_{CUP}-*ADE5,7*, the *CUP1* promoter, being inducible mainly by Cu(II) ions [30], leverages the adenine monophosphate pathway altered by *ADE2* deletion to enable intracellular accumulation of red pigments in the yeast in high Cu(II) and high O_2 environments, resulting in visible color changes commensurate with Cu(II) concentration. The modified *S. cerevisiae* cells were immobilized in alginate beads and the accumulation of red pigment was found to accurately correlate with Cu(II) concentrations within the 1–100 µM range.

A *Cupriavidus metallidurans* CH34-based biosensor was developed to quantify Cu(II) ions in aquatic environments through the expression of yellow betaxanthin pigments [31]. *C. metallidurans* possesses the copSR regulatory system, which grants the microorganism the ability to thrive in environments with high copper levels. To produce an effective biosensor, different promoters of the *cop* cluster identified in the organism's genome—*PcopT*, *PcopQ*, *PcopH*, *PcopA*, and *PcopM*—were first evaluated in red fluorescent protein biosynthesis assays. To identify the ideal promoter, plasmid backbones bearing *copS-copR* sequences under the control of native promotor and one of the candidate *cop* promoters driving the expression of reporter gene *rfp* were each transformed into *C. metallidurans*.

3.2. Response of WCBs to Cadmium

Colorimetric WCBs can, in low-resource areas, supplant more complicated and voluminous equipment and enable in situ analyses. Cadmium (Cd) constitutes a source of considerable disruptions within ecosystems given its bioaccumulation, toxicity, and persistence in the environment [32]. A host of WCBs have been devised to detect Cd among other heavy metals and include fluorescent, chemiluminescent, and bioluminescent reporters. A number of Cd-specific metalloregulators have been used in various biosensors to reveal the presence of Cd. Most saliently in the context of WCBs, *CadR*, which is categorized under the *MerR* subfamily of metal-ion-sensing transcriptional regulators with variable specificities and was characterized in *Pseudomonas aeruginosa* [33], regulates its own transcription as well as that of a Cd efflux P-type ATPase *CadA*, thus making microbial species resistant to high concentrations of the metal.

The Phytoene dehydrogenase (CrtI)-enabled synthesis of red carotenoid pigment deinoxanthin from colorless substrate in *Deinococcus radiodurans* was leveraged to produce a Cd-selective biosensor [10]. In this instance, *D. radiodurans* was engineered to exclusively produce deinoxanthin in the presence of Cd(II). To achieve this, the researchers utilized a colorless strain of *D. radiodurans* dubbed KDH018, whose *crtI* gene had been previously deleted, as the WCB chassis. To use red deinoxanthin as a reporter, and using *E. coli* for all genetic manipulations, *crtI* was cloned from chromosomal DNA of wild-type *D. radiodurans* onto a pRADZ3 *E. coli* to *D. radiodurans* shuttle vector, thus generating pRADI [34].

While microorganisms with inherent Cd(II) resistance are conspicuous candidates to explore in this context, model organisms such as *E. coli* can also be utilized to this end. To detect Cd(II) ions in environmental water with relatively high specificity, a violacein-producing colorimetric biosensor was devised using *E. coli* as a chassis [35]. The sensory module consisted of the metalloregulator *cadR* gene, originally characterized in *Pseudomonas putida* [36], and its divergent *cad* promoter, whereas the reporter module consisted of a synthetic *vioABCDE* gene cluster, characterized in *Chromobacterium violaceum* [37], enabling violacein synthesis (**Figure 2**) [38].

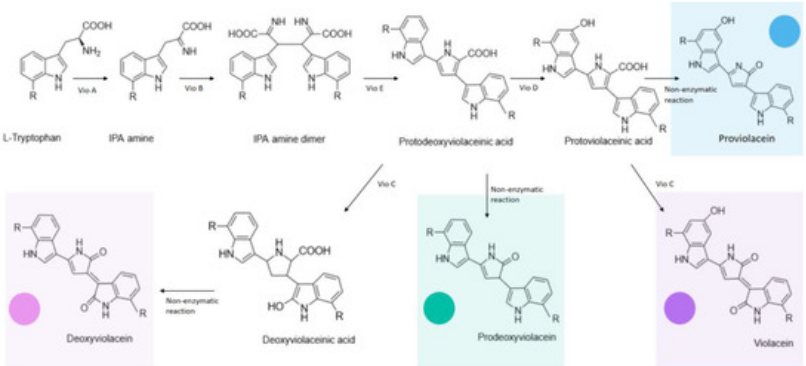

Figure 2. Enzymatic and non-enzymatic reactions implicated in the violacein biosynthetic pathway encoded by the vioABCDE cluster, as well as the different colored products they yield.

3.3. Response of WCBs to Lead

Environmental lead (Pb) is either naturally occurring or resulting from anthropogenic activity given its use in automotive batteries, telecommunications, construction, and its erstwhile use in pipes and paints among others. The toxicity threshold of lead to humans and animals is quite low, with chronic exposure resulting in anemia, neurotoxicity, and severe renal damage [39]. *PbrR*, a transcriptional regulator and part of the *MerR* subfamily, mediates the Pb(II)-induced transcription from its divergent promoter and regulates the *pbr* operon as part of some microorganisms' lead detoxification systems [40]. The *pbr* operon was first identified in *Cupriavidus metallidurans* CH34 and encodes a particularly comprehensive Pb resistance mechanism which entails transport, efflux, sequestration, precipitation, and biomineralization [41]. As such, components from the *pbr* operon can and have been successfully utilized in the conception of Pb-sensing WCBs producing various readouts [42][43][44].

The indigoidine reporter module described earlier was also utilized in conjunction with a Pb(II) sensory module comprised of the *PbrR* transcription regulator and its divergent promoter [45]. The DNA fragments bearing *pbrR* the *pbrR*-divergent *pbr* promoter, borne on the pT-Ppbr plasmid constructed in an earlier study [46], were amplified and cloned into the pT7lac-ind plasmid to yield pPpbr-ind, which was then transformed into *E. coli* TOP10 competent cells. The output signal is reliably produced 3 h post-incubation with the inductor present, and it can be spectrophotometrically assessed at λ = 600 nm to derive the concentration of bioavailable Pb(II). The authors noted that WCBs harvested at the lag phase were more apt at detecting Pb within the 0.13–4.17 µM range, whereas those harvested at the exponential phase produced the best results within the 0.26–8.3 µM range. While a color change detectable by the human eye can only be observed at 4.17 µM, the method is still conducive to a rapid and reliable spectrophotometric assessment of Pb content in waters suspected of contamination.

Pb(II)-dependent metalloregulator protein PbrR was also used as a component of a sensory module in an *E. coli* TOP10-based WCB leveraging the violacein biosynthetic pathway [38]. The violacein synthetic pathway was first cloned onto a pET21a plasmid to yield pET-vio. Subsequently, the DNA fragment containing gene *pbrR* and its divergent promoter *Ppbr*, borne on the pT-Ppbr plasmid constructed in an earlier study [46], was PCR-amplified and cloned onto the pET-vio plasmid to yield pPpbr-vio used to transform *E. coli* TOP10. Three hours post-incubation in Pb(II)-containing media, the produced violacein could be extracted using butanol and quantified at λ = 490 nm. A linear relationship between Pb(II) concentration and violacein concentration was observed within the 0.1875–1.5 mM range. Beyond the upper limit of the range, the sensor exhibited qualitative properties up to 24 µM, which was the highest tested concentration. An advantage proffered by the use of violacein in this sensor was the ability to visually detect amounts of Pb(II) in the medium at levels below 0.1875 mM.

Building upon this previously mentioned work, the researchers resorted to subcloning techniques to generate plasmids pPpbr-vioABE, pPpbr-vioABDE, and pPpbr-vioABCE from pPpbr-vio [47]. Each of the plasmids including pPpbr-vio was transformed into *E. coli* TOP10 to create four WCBs relying on different reporter pigments. The violacein- and deoxyviolacein-producing strains were retained for later assays given that the expected pigment output was produced as opposed to what was observed with the remaining strains. This occurrence can be attributed to the instability of prodeoxyviolacein and proviolacein intermediates as well as the highly branched metabolic pathway culminating in violacein synthesis [48]. The deoxyviolacein-based biosensor displayed considerable efficacy and a narrow dose-dependent response across a broad range of Pb(II) concentrations spanning 2.93–6000 nM, whereas the violacein-based one exhibited a less narrow dose-dependent response between 2.93 nM and 750 nM.

3.4. Response of WCBs to Mercury

The highly toxic nature of bioaccumulative mercury (Hg) coupled with its relative abundance in the environment make it a veritable hazard to public health. Several bacteria have developed resistance mechanisms to the metal. These detoxification mechanisms, enabled by an inducible set of genes arranged in a single *MerR* operon under the control of the metal-sensing MerR protein [49], have been exploited in a number of contexts to create crucial biosensors enabling the safeguarding of human and animal health.

The polyvalence of the indigoidine reporter module is further evinced in its robustness at signaling the presence of Hg(II) in an *E. coli*-based WCB [45]. This was achieved by coupling the reporter module with a sensor module consisting of the *MerR* gene encoding the metalloregulator protein MerR and its divergent *mer* promoter region, which had been synthetically produced and introduced into a pET-21a plasmid to generate Ppmer [50]. The DNA fragments contained in Ppmer were PCR amplified and cloned into the previously referenced pT7lac-ind plasmid to generate pPmer-ind, which was then transformed into *E. coli* TOP10. WCBs harvested at the exponential phase of bacterial development were found to reliably signal and quantify Hg(II), which induced a dose-responsive indigoidine biosynthesis at a concentration range

spanning 0.008–0.52 µM, with the color of the pigment exceeding the human eye detection threshold at Hg(II) concentrations above 0.033 µM.

The violacein reporter developed by Hui et al. and utilized to detect Pb in an earlier work [38] was repurposed in the creation of a Hg(II) biosensor [51]. The Ppmer sensor module which was constructed by Zhang et al. [50] was also utilized in this undertaking. To assemble the sensory and reporter apparatuses, the researchers PCR amplified the DNA fragment containing *merR* gene and its divergent *mer* promoter from the Ppmer plasmid and cloned them into pET-vio to generate pPmer-vio. The recombinant plasmid was transformed into *E. coli* TOP10 to yield a Hg-sensing WCB which would respond to Hg by producing the violet pigment violacein. A dependable readout from WCB cells harvested during the exponential phase was obtained 5 h post-induction, and it exhibited a dose-dependent pigment-based response to Hg(II) in the range of 0.78–12.5 µM. Beyond this upper limit, violacein synthesis reportedly decreased as a result of toxicity. Inductive amounts of Hg(II) equal to and beyond 6.25 µM incurred the production of enough pigment to be detected by the human eye post-extraction using butanol. Sensors harvested at the lag phase exhibited a dose-dependent response to Hg(II) within the 0–0.12 µM range, thus allowing for the quantification of more minute amounts of Hg(II). Violacein was visible with the human eye post-extraction at Hg(II) concentrations withing the 0.006–0.098 µM range and the intensity of the violet color diminished past the Hg(II) concentration of 0.024 µM due to cytotoxic effects.

Transcription regulator MerR has also been used as a sensor module in a *P. aeruginosa* WCB employing reporter genes *phzM* and *phzS*, encoding for the enzymes methyltransferase and flavin-containing monooxygenase, respectively [52]. These enzymes catalyze the synthesis of pyomelanin [53], a red–brown pigment with potent antioxidative properties protecting microorganisms from oxidative stress [54]. A recombinant plasmid carrying *merR* under the control of native promoter and the *phzM* and *phzS* genes under the control of the *mer* promoter, was transformed into *P. aeruginosa* PAO1. The WCB worked well within a broad pH range, proved to be highly selective by responding poorly to other metal ions and produced a dose-dependent response to Hg(II) between 25 and 1000 nM. Prior to spectrophotometric quantification of pyocyanin to derive Hg(II) concentrations, the hydrophobic pigment must be extracted from the cells using chloroform and hydrochloric acid.

3.5. Response of WCBs to Arsenic

Over one hundred million people are effected by arsenic (As) water contamination across the world and are thus prone to developing skin lesions and gastrointestinal distress in case of chronic exposure to low doses, although high concentrations pose a much greater toxicity risk to human health [55]. A number of WCBs were elaborated utilizing the *ars* operon, which consists of two regulatory genes (*arsR* and *arsD*) and three structural genes (*arsA*, *arsB*, and *arsC*) and contributes to arsenite and arsenate resistance by detoxifying the cell [56]. Highly contaminated areas include Indian and Bangladeshi industrial zones, and remediation must be enabled by access to cheap and dependable technologies.

The bright colors of carotenoid pigments spheroiden and spheroidenone were exploited in the creation of an arsenite biosensor [57]. In its wild form, photosynthetic bacterium *Rhodovulum sulfidophilum* produces the red pigment spheroidenone in semi-aerobic conditions via the spheroidone pathway enabled by genes *crtF* and *crtA*. The former encodes for O-methyltransferase which acts upon the C-1 hydroxy group of demethylspheroidene resulting in the synthesis of yellow spheroiden, and the latter codes for a monooxygenase subjecting the spheroiden produced in the earlier step to a C-2 ketolation thus yielding red spheroidenone. As such, a mutant strain with the *crtA* gene deleted, such as *R. sulfidophilum* CDM2, would accumulate yellow pigments. To create the arsenite sensor, Fujimoto et al. relied on a strategy predicated on a color shift from yellow to red using *R. sulfidophilum* CDM2. To that end, they constructed a reporter module consisting of a promoter-less fragment of the *crtA* gene which was cloned onto a broad-host-range plasmid pRK415 together with the *E. coli*-derived sensory module comprising the arsenite responsive *E. coli* DNA fragment containing the operator/promoter of the *ars* operon (*O/pars*) as well as the the *arsR* repressor. The recombinant plasmid, pSENSE-As, was transformed into *E. coli* S17-1 and transferred into *R. sulfidophilum* CDM2 through conjugation. Preliminary assessments confirmed that *E. coli O/Pars* was recognizable by CDM2 RNA polymerase and that no transcription repression by an endogenous protein occurred in CDM2.

4. Biomonitoring and Control

4.1. High-Level Producer Detection

Lysine is an amino acid with considerable importance in the context of human and animal nutrition and is the second most abundantly produced essential nutrient worldwide [58][59]. *Corynebacterium glutamicum* is an effective production platform of L-lysine and other amino acids, and engineered strains have been turned into industrial workhorses specially created for this purpose [60].

A notable drawback on pSenLys-based sensors is their inability to accurately report the overproduction of a specific amino acid among lysine, histidine, and arginine. The non-specificity of pSenLys prompted the exploration of different avenues. Liu et al. detailed the development of a *C. glutamicum* based colorimetric WCB with greater lysine specificity, utilizing lycopene as a reporter pigment [11]. In response to L-Lys, *LysG* activates the expression of *crtI* encoding phytoene desaturase which then catalyzes the production of lycopene with the characteristic red color. To that end, a *C. glutamicum* mutant strain, deficient of the carotenogenic gene cluster *crtIYe/fEb*, *crtB2I21/2*, and *LysEG* was first generated as a sensor chassis. To construct the plasmid, transcriptional regulator *lysG*; its binding site region *lysE* promoter; and the phytoene desaturase gene *crtI* were amplified using the genome of *C. glutamicum* as a template. The expression cassette was fused using overlap extension PCR and cloned into a pTRCmob vector plasmid dubbed pSensorI. The sensor plasmid was transformed into *C. glutamicum* WT-*ΔlysEGΔcrtIYEbB22* and this transformed strain bearing pSensorI served as a control as other optimizations were implemented. To remedy the poor specificity of *lysG* and diminish false positives induced by docking of L-histidine and L-arginine, *LysG* was subjected to site-directed saturation mutagenesis to screen for mutants with reduced affinity to L-histidine and L-arginine. Substitutions at positions 123 and 125—where L-glutamate was substituted with L-L-tyrosine and where L-glutamate was replaced with L-alanine, respectively—were found to confer the modified binding site *lysG** a drastically reduced affinity to L-arginine and L-histidine and an uncompromised colorimetric linear response to L-lysine. To increase the range of dose-responsiveness up from the reported 40 mM limit, the researchers resorted to promoter engineering of *pLysE* and 5 promoters were screened. Promoter *pLysE-3* was selected as the most apt candidate and was found to engender an increased range of responsiveness of up to 300 mM. To enhance the performance of the sensor from a color-rendering standpoint and thus facilitate overproducer detection, the *CrtR* transcriptional regulator, which is known to repress the *crt* operon [61], was deleted by electroporating a suicide plasmid into WT-*ΔlysEGΔcrtIYEbB2* to construct WT-*ΔlysEGΔcrtIYEbB2R*.

4.2. Pathogen Detection

N-acyl homoserine lactone (AHL) is a signal molecule utilized by a number of gram-negative bacteria for cell-to-cell communication as part of the quorum sensing mechanism. The utility of WCBs leveraging the quorum sensing apparatus of microorganisms has a number of benefits namely signaling the presence of possibly pathogenic species [62], monitoring bacterial populations in bioreactor settings [63], and modulating the microbial composition of a medium [64].

N-butyryl-L-homoserine lactone (BHL) is an AHL and a small diffusible signaling molecule implicated in quorum sensing, the control of gene expression, and cellular metabolism [65]. To detect minute amounts of BHL within a wide concentration ambit, Yong and Zhong developed a *P. aeruginosa*-based biosensor [66]. The researchers used strain *P. aeruginosa* CGMCC 1.860, which is naturally capable of producing blue–green pigments upon detecting BHL. This is achieved through the RhlR-RhlI quorum-sensing system, which comprises the transcription activator protein RhlR and the BHL synthase RhlI [67]. To create a biosensor, the researchers deleted the *rhlIR* gene cluster, thus creating *P. aeruginosa ΔrhlIR*, and overexpressed *rhlR* through multi-copy plasmids. As such, the bacteria regained the capacity of sensing BHL while avoiding the production of the analyte by endogenous activity. The recombinant biosensor strain is thus capable of producing the pigment upon sensing of exogenous BHL which can diffuse in the cell and be recognized by the RhlR regulator. Upon BHL binding, this transcription regulator activates the expression of pigment synthases. The resulting WCB whose pigment output can be extracted using chloroform and quantified at $\lambda = 299$ nm exhibited dose-dependent pigment production within the 0.11–49.7 μM AHL range.

4.3. Micronutrient Quantification

Micronutrient deficiencies are significant concern of global ambit although gauging the veritable magnitude of the issue remains challenging [68]. In remote settings and in impoverished parts of the world, access to reliable testing is limited due to elevated costs and logistical difficulties. Colorimetric WCBs as part of field-ready kits can be handled by agents with minimal training to provide in situ testing in remote areas, identify micronutrient deficiencies, and help remedy health complications quickly and reliably.

Zinc is an essential micronutrient; deficiencies have incurred public health burdens of significant magnitude, and one billion people across the world are presumed to be at risk of zinc deficiency [69]. Efforts to provide access to impoverished and remote areas of the world have yielded the development of colorimetric biosensors.

In the context of early efforts to develop a colorimetric biosensor compatible with zinc serum levels, Watstein and Styczynski generated an *E. coli*-DH10B-based sensor capable of producing three different reporters: violacein, lycopene, and β-carotene [12]. A violacein operon was cloned onto a plasmid bearing zinc-responsive transcription regulator *ZntR*, proprietary ribosomal binding sites, and the gene encoding the Zur metalloregulator protein, which acts as a zinc-

responsive repressor. Gene cluster *vioABCDE* was placed under the control of a *PznuC* repressor actuated by Zur–zinc complexes.

5. Conclusions

The development of whole-cell biosensors keeps up with the pace of broad ranging advancements in genetic engineering and practically puts to use novel approaches stemming from recent advancements. As such, it allows for more nuance to materialize and for a greater understanding of processes and mechanisms to be gleaned. WCBs are a highly versatile platform enabling the development of accessible and inexpensive analytical devices which, in some circumstances, replace their less portable laboratory analogues [70]. They can be adapted to a considerable range of analytes that grows as more regulatory as well as biosynthetic pathways are characterized. Moreover, they benefit from a wide selection of thoroughly understood microbial chassis to transform based on the biosensor's purpose. Indeed, the caveat of these assessments being conducted in highly controlled settings must be borne in mind. In effect, they were mostly undertaken using solutions of known analyte concentrations and compositions. Their effectiveness in the field may be less pronounced due to a host of causes including the general complexity of natural matrices which may contain compounds of bacterial origin or otherwise which may affect their performance or result in false positives and negatives.

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