Development of a bioluminescence-based whole-cell biosensor for air pollution monitoring.

Research article written as part of the 2016 iGEM Competition by the iGEM IONIS Team

Abstract

Air pollution is a major issue, especially in urban areas where levels of pollutants are constantly on the rise. The concentration of some pollutants like Volatile Organic Compounds (VOCs) is poorly measured even though they are known to have multiple harmful effects, such as carcinogenicity, reproductive toxicity, and environmental toxicity. This is mostly due to a lack of appropriate detection technology. Existing tools present major flaws, such as long exposition times or important device size, that prevents any efficient VOC detection. In this article, we propose a new technology for VOC detection. This technology, based on a whole-cell biosensor, present numerous advantages over the existing devices and is especially suitable for on-field measurements. Unlike existing biosensor reporters, we chose to use bioluminescence as the quantifiable reporter signal. Luciferase genes have been extensively used as an imaging tool but only few articles report their use as a reporter gene. We engineered *E. coli* strains to constitutively produce the XylR protein, a transcription factor capable of binding VOCs. Once bound to these compounds, the XylR-VOCs complex activates the Pu promoter. This promoter triggers the expression of the luciferase reporter gene. Engineered *E.coli* cells were treated with several concentrations of toluene and the bioluminescence intensity of the cells was measured. The bioluminescence intensity of the cells was proportional with the amount of toluene added to the cell culture medium. To better investigate the reliability of the obtained results, the effect of toluene on cell survival was assessed along with the best possible measurement time after toluene exposure. The on-field advantages of whole-cell biosensors were demonstrated through the construction of a prototype drone. The drone acts as a mobile detection platform for sampling and mapping purposes. In conclusion, we developed a proof-of concept innovative biosensor and proved it to be functional, opening the way for further work on bioluminescent-based biosensors.

Comments

jgoupil: This abstract, while very informative, is a little on the long side. I think there are some technical details here about how you went about making your biosensor that can be either omitted or condensed. I'm particularly thinking about the following section: "We engineered E. coli strains to constitutively produce the XylR protein, a transcription factor capable of binding VOCs. Once bound to these compounds, the XylR-VOCs complex activates the Pu promoter. This promoter triggers the expression of the luciferase reporter gene. Engineered E.coli cells were treated with several concentrations of toluene and the bioluminescence intensity of the cells was measured. The bioluminescence intensity of the cells was proportional with the amount of toluene added to the cell culture medium."

Clément Lapierre: Hello. First of all, thank you for your comment ! This construction is at the center of our project, it is its very core, which is why we put it in the abstract: we wanted to clearly explain what we were doing.

renberg: Typo: "only few articles reporter their use as a reporter gene" should be only few articles report their use as a reporter gene

Clément Lapierre: Oops ! We took care of it. Thank you very much for noticing !

Keywords: VOC, iGEM, Synthetic Biology, Bioluminescence, Biosensor

Introduction

Air pollution is an ever-increasing concern, especially in urban areas. Air pollution is generally described as the presence of gas and particles in the outside air with harmful impact on human health and/or on the environment. Air pollution is a complex phenomenon as pollutants are numerous, originate from many sources and as many factors impact air pollution. Pollutants may come from natural phenomena (volcanic eruptions, organic matter decomposition, forest fires) as well as human activities (industry, transport, farming and residential heating) [1]. Among the most harmful pollutants are the Volatile Organic Compounds (VOCs). VOCs are organic chemicals with a high vapor pressure (0.01kPA) at room temperature (293.15K = 20°C). Their high vapor pressure results from a low boiling point, which causes large numbers of molecules to evaporate from the liquid or sublimate from the solid form of the compound and enter the ambient air, a trait known as volatility [1] [2]. VOCs have been more and more investigated over the last years. These primary pollutants originate from fuel evaporation, car traffic, industrial processes, heating system in residential area, domestic use of solvent but also from vegetation. Although they represent only 2% of the pollutants found in the air [2], VOC, especially the BTEX (Benzene, Toluene, Ethylbenzene, Xylene), were shown to have many harmful effects, such as carcinogenicity, reproductive toxicity, and multiple systemic effects [3]. They also acidify water, soil, and impact vegetal growth [3] and play a role in secondary particle formation and ozone [4][5]. The estimated cost of these premature deaths in WHO countries is $ 1.6 trillion per year. [5]

To protect populations and the environment against those harmful effects, the concentrations of those pollutant needs to be monitored and a threshold of 5 µg/m3 needs to be respected.

The number of different pollutant molecules is so important that no universal detection technology is possible. Various technologies exist for every pollutant, and they have mainly been classified in two main groups: Active and Passive. A study of the existing methods for VOCs detection can be found in Table 1. As we can see in Table 1, the existing technology for VOCs detection are either not reliable or precise but costly, energy consuming and requiring big machines. It has been proved that the biggest pollution variations are recorded within a 20m range around a highway [2], hence proving the need for a detection technology made of a small device, adapted to small-scale pollution measurements. Biotechnology and synthetic biology are promising alternatives to existing technologies as they provide small measurements devices.

Method	Method type	Advantages	Disadvantages	Method duration	Materials	Associated uncertainty	Cost
Continuous analysis scanner* Ex Mini RAE 3000	Physical	Quick results	Electrical +gas Alimentation, difficult calibration, Costly maintenance	15 min	Device	11 % (5µg/m3)	3800- 30 000 €
Integrated measure by active sampling **	Physical	Important air volume sampling, Any water molecule trapping	Very long measurement time	24 hours to 1 week (according to the adsorbent)	2 systems: - portative - transportable power sector (sequential sampling)	Concentrations associated uncertainty (ug) for toluene: 14, o-xylene: 23, m/p-xylene : 28	Commercialized device + or - sophisticated and costly (1000-20000€)
Integrated measure by passive sampling ***	Physical	Autonomy, quiet and easy to use	Radial: superior to 10 µg/m3 (qualitative measure), Influence of interference.	Sampling duration: 7 to 15 days	Tubes with axial or radial diffusion	Radial: 22% (7 days: 5µg/m3) Axial : 30%	
LIDAR: Light Detection and Ranging	Active; physical	Sensitive, Study of pollutants spreading	20-45 Kg, around the size of a small fridge				200k €
Chemical analysis	Chemical	Specific compound identification	Any information on compound biologic activity, Not environment friendly				
Whole cell	Biological; Enzyme	Very sensible, Large scale analysis	Enzyme purification required, Good knowledge of enzyme properties	Result within hours	Variable	Low	Expensive
	Biological; passive, Bacteria	**Easy to use, Large scale analysis numerous organisms usable, good for aerial environment**	**Ethic, Sterile work, Low toxicity of the detected compound is necessary**	**Results in hours/days**	**Variable**	**Improvable sensitivity by modifying the genetic system**	**Not expensive**
	Biological; passive; yeast	Large scale analysis	Ethic, Sterile work Less known than bacteria Slower growth and metabolism	Results in hours/days	Variable	N.A	Not expensive
	Biological; passive; Algae	Large scale analysis, Numerous organisms usable, good for marine environment	Special luminosity, Ethic Adapted for marine environment Less known than bacteria	Results hours/days	Variable	N.A	Not expensive

Table 1. Existing VOC measurement methods. [7][8][9][10][11]

Comments

theresa: I think this should be moved up in the text to where it is referenced so it is easier to understand in context.

Clément Lapierre: Done ! It is indeed simpler. Thank you for your review !

In this article, we aimed to develop a whole-cell biosensor able to efficiently and rapidly quantify the toluene concentration (chosen as our main test molecule for safety reasons). Whole-cell biosensors have been proven to be especially suitable to on-field application[7][8][9][10]. We used the transcription factor XylR, from the Tol plasmid of *Pseudomonas Putida*, known to bind toluene and other pollutants from the BTEX family [11][12]. Upon binding to toluene, the XylR-toluene complex can activate the Pu promoter[13][14]. The efficiency of XylR to bind toluene has been proven by previous iGEM team such as the Peking 2013 iGEM Team[17]. It is important to note that this article focuses on the development and use of the biosensor system and does not investigate additional XylR properties, such as the reversibility of the XylR/Toluene binding or the XylR/Toluene complex elimination.

We engineered *E.coli* bacteria to constitutively produce the XylR protein and express the reporter gene Gaussia luciferase [18][19] under the control of the Pu promoter. Once produced, the Gaussia luciferase oxidizes its substrate (coelenterazine) to produce bioluminescence. Bioluminescence presents several advantages over chromoproteins or fluorescence, such as an increased sensitivity, and allows quantitative measurement of the reporter signal[19][20][21].

Using a mobile detection platform, such biosensor could enable quick and precise on-field measurement of air pollution.

Materials and methods

Chassis

The *Escherichia Coli* strain DH5α was used as chassis for the plasmids. Indeed, this strain grows easily and has several mutations that make it an excellent choice for cloning procedures with a high efficiency transformation. All designed plasmids have been optimized for *E. Coli*. Moreover, *E. Coli* is a model organism, entirely sequenced.

Plasmids

The plasmid backbone mainly serves as support for the propagation of the biobrick part. It includes the origin of replication and the antibiotic resistance marker. The pSB1C3 plasmid, a high copy number assembly plasmid, was used as a vector of 2070 pb, because it is the most used type of backbone to assemble DNA fragments. This plasmid backbone includes the chloramphenicol resistance marker and a high copy replication origin, which allows a high copy number per cell, which facilitates DNA purification.

BioBrick RFC [10] assembly standard

For the assembly of DNA fragments the BioBrick RFC [10] assembly standard was used because it is the standard of the IGEM competition and most of the parts available on the registry match this standard. The BioBrick RFC [10] assembly standard is based on the use of a prefix and a suffix placed at the extremities of each part to be assembled in order to obtain standard BioBricks that are compatible and thus can be easily assembled (Figure 1). Prefix and suffix, each one, includes two different restriction sites. *EcoR*I and *Xba*I in the prefix. *Spe*I and *Pst*I in the suffix. The use of this assembling method leaves a scar between the 2 assembled DNA fragments.

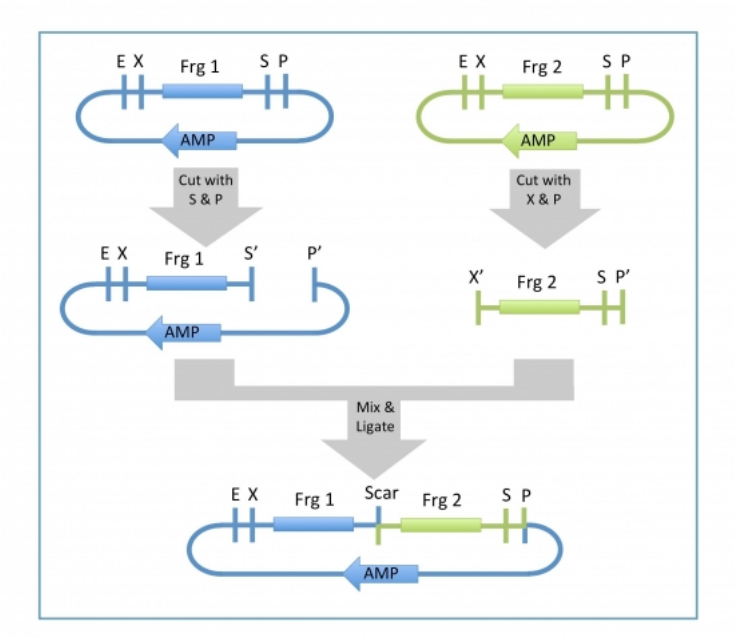

Figure 1. Assembling method with RFC[10] standard. Fragment 1 is digested using SpeI et PstI while fragment 2 is digested using XbaI and PstI. During the ligation, fragment 2 is inserted after fragment 1 thanks to the matching DNA extremities left by the enzyme couple SpeI/XbaI.

Primers Design

Ten primers (Table 2) were designed using the Geneious software to amplify the DNA fragments that compose our plasmids. Six primers (Table 3) were designed using the Geneious software to sequence plasmids. The primers were synthetized by Eurofins.

Name	Binding site	Direction	Length [bp]	%GC	Tm [°C]	Ta [°C]	Sequence (5'->3')
A12	Prefix	Forward	27	55.6%	68	NA	ATCTGGAATTCGCGGCCGCTTCTAGAG
A13	Suffix	Reverse	26	57.7%	68	NA	TACTAGTAGCGGCCGCTGCAGTCCATG
SDM-F	Start codon of XylR	Forward	28	29%	56	57° C	GACTACTAGATGTCACTTATTTATAAAC
SDM-R	Before start codon of XylR	Reverse	18	44%	58		CTTACCGCTAATTCTACG
AHis-tag	End of XylR gene	Reverse	60	58.3	60.8	NA	CTACTAGTGCTAGTGGTGATGGTGATGATGCCCCCCACGCCCCGTAGCCTTGACTGACAA
BBA-R	End of the first double terminator of BB123	Reverse	58	50%	>75	NA	ATGGACTGCAGCGGCCGCTACTAGTACGCGCTTTCCCGGGCTCTAGTAAAATAATAAA
BBB-F	Beginning of Pu	Forward	47	59.6%	>75	NA	ATCTGGAATTCGCGGCCGCTTCTAGAGCCCGGGAAAGCGCGATGAAC
Rev-C4	End of RBS	Reverse	48	50%	54.8	NA	ATGGACTGCAGCGGCCGCTACTAGTAGTCCTTACCGCTAATTCTACGC
Fw-Gluc	Beginning of Gluc gene	Forward	48	47.6%	52.4	NA	ATCTGGAATTCGCGGCCGCTTCTAGAGGGACTACTAGATGGGAGTGAA
Rev-Gluc	End of Gluc gene	Reverse	47	52.4%	54.4	NA	ATGGACTGCAGCGGCCGCTACTAGTACTATTAATCACCACCGGCACC

Table 2. Primers used to create the plasmids

Comments

theresa: Again I think this table would make more sense up under the primer design section along with table 3. This way it is easily seen where it is referenced in the text.

Clément Lapierre: We modified the table position accordingly, thank you very much !

Name	Direction	Length [bp]	%GC	Tm [°C]	Ta [°C]	Sequence (5'->3')
VF2	Forward	20	50%	57.3	NA	TGCCACCTGACGTCTAAGAA
VR	Reverse	20	50%	57.3	NA	ATTACCGCCTTTGAGTGAGC
XylR-1	Forward	27	48.1%	65	NA	GGTGGATCATCCCGATACTAGAGAAAG
XylR-2	Forward	25	52%	64.6	NA	GGATACGCAAGTGGATACGGTAGTG
XylR-3	Forward	26	46.2%	63.2	NA	CTTGGATGAGGTCATAGAACTTACGC
XylR-4	Forward	20	55%	59.4	NA	CACGTTTGCTTGGCTTGACC

Table 3. Primers used to sequence the plasmids.

Toluene evaporation test

The toluene is known to be a volatile compound. Therefore, it was decided to determine its rate of evaporation before proceeding to any subsequent manipulation with it. This will determine the care that has been to take when handling it as well as the time the stock solution will be kept. 1.2mL of pure toluene (244511, Sigma-Aldrich) was added in a 1.5mL Eppendorfs tube. The tube was left open under a chemical hood and its weight was determined every 30 min for 6 h.

Toluene survival test

A large range of concentrations (from 5ng/L to 5mg/L) was tested in order to evaluate the overall impact of toluene on the *E.coli* DH5α cell growth. 5ng/L is a concentration close to environmental toluene concentrations. An overnight culture of bacteria transformed with our biosensor was realized. 300µL of this overnight culture was inoculated into 150mL of LB containing chloramphenicol at 25ng/mL. This culture was homogenized and separated into several falcon tubes. Toluene was then added to the culture medium to reach desired concentrations and the sample's OD600 was checked every hour during 8 h. Three replicates of each concentration were done to ensure statistical results.

Biosensor characterization

It was decided to test several concentrations of toluene (0ng/L, 10ng/L, 100ng/L, 10µg/L and 10mg/L). Bioluminescence assays were realized several times after toluene addition (1h, 3h, 4h30 and 5h30). Bacteria transformed with the biosensor used in each assay come from the same culture (OD = 0.1). This culture was mixed and divided in 50 mL falcons. Toluene was then added in each Falcon. Bioluminescence assays were realized quickly after substrate addition and the bioluminescence intensity was measured using Mithras² LB 943 Monochromator Multimode Reader. This machine was kindly lent by Berthold Company for 3 days in order to let us realize the bioluminescence measurement. In addition to bioluminescence intensity, sample's OD was also measured.

All tests were realized in triplicates. Several negative controls were realized:

- Measurement of LB bioluminescence intensity to determine the background noise.

- Measurement of LB+toluene bioluminescence intensity to determine toluene effect on the bioluminescent intensity.

- Measurement of the bioluminescence intensity of bacteria transformed with a genetic construction that do not contain the Gaussia luciferase gene (Pr-XylR).

A positive control using Gluc coding device with Pr (BBa_K2023007) could have been made during the three days of measurement to test substrate efficiency and luminometer parameters. However, this BioBrick was created afterwards. This BioBrick will serve as a future control for all future bioluminescence assays to ensure the substrate efficiency.

Results

Toluene evaporation test

Toluene was experimentally proven to be a volatile compound: left open under a chemical hood, 419 mg of pure toluene have evaporated from the tube within 6 hours. These results show that toluene evaporates with a rate of 72 mg per hour. However, we supposed that this rate could have been increased by the action of the chemical hood. Therefore, care has to be taken when manipulating it. Each tube containing toluene has to be open under a chemical hood and any toluene tubes must be left open. Care has to be taken when adding toluene to bacteria culture medium.

Comments

theresa: Rewording the end of this paragraph about safety with the toluene may help get your point across better. Right now it is a little confusing because it states tubes containing toluene must be open under a hood and then adds that toluene tubes must be left open which you already said.

Clément Lapierre: We indeed modified the text since then, thank you for your comment !

The stock solution of toluene will be prepared again for each replicate. With these results, we can then test the mortality of *E.Coli* DH5α when exposed to several toluene concentrations.

Time	t_i = 0 min	t_i = 30 min	t_i = 60 min	t_i = 90 min	t_i = 120 min	t_i = 150 min	t_i = 180 min	t_i = 210 min	t_i = 240 min	t_i = 270 min	t_i = 300 min	t_i = 330 min	t_i = 360 min
Weight	1051 mg	979.6 mg	937.7 mg	889.4 mg	836.5 mg	805.7 mg	778.2 mg	766.1 mg	732.4 mg	717.2 mg	683.7 mg	656 mg	632.5 mg

Table 4. Variation of the liquid toluene weight in function of time.

Survival test to toluene

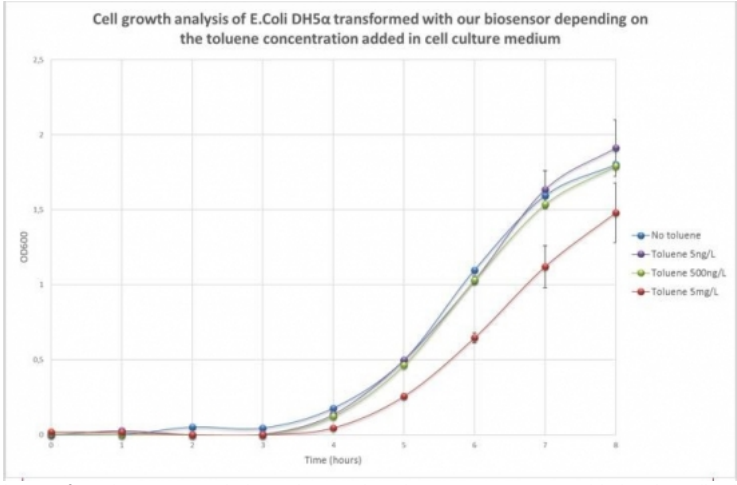

Figure 2. Evolution of E.coli DH5α growth depending on the toluene concentration added in the cell culture medium

Comments

theresa: I think this should be up closer to the paragraph it is referenced in.

Clément Lapierre: This has been modifies, thank you very much for pointing it out !

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The Figure 2 shows the evolution of sample optic density (OD600) according to the time and the toluene concentration added in cell culture medium. The curve shows normal growth profile with a lag phase of three hours and then an exponential phase for all samples whether the toluene concentration added in the cell culture medium.

Toluene addition at a concentration of 5ng/L and 500ng/L did not significantly affect cell development. The growth curve shows the same profile than cells grown without toluene. At a concentration of 5mg/L of toluene in the medium, an effect on cell development can be noticed. Four hours after toluene addition, the OD600 of cell grown in 5mg/L of toluene increased more slowly than the OD600 of cell grown without toluene.

In compliance with those results, bioluminescence tests were realized with a toluene concentration in the culture medium comprised between 0 and 10 mg/L.

Biosensor characterization

Preliminary considerations

Toluene does not produce background noise. The effect of toluene addition to cell culture medium on bioluminescence intensity was determined. As shown on Figure 3A the bioluminescence intensity of the LB with toluene at 10 mg/L (47.71 RLU) does not significantly differ from the bioluminescence intensity of the LB without toluene (48.93 RLU). The small recorded signal was due to the substrate added in our sample. Therefore, toluene addition in samples will not impact the bioluminescence intensity.

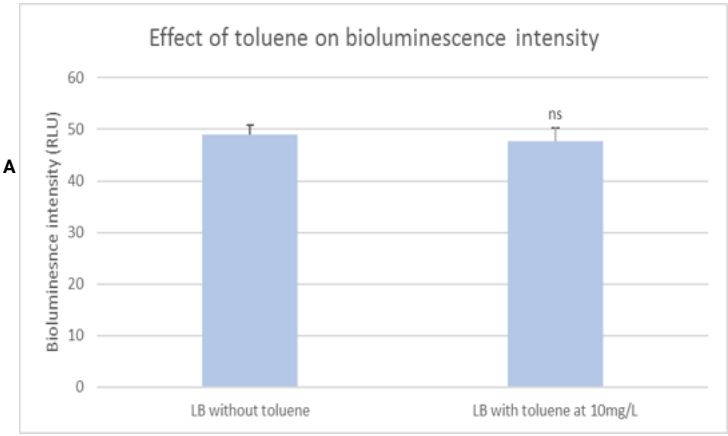
***E.coli* Bacteria have little background noise.** Bacteria transformed with a genetic construction that does not contain Gaussia luciferase gene were used in this study. The background noise of *E.coli* cells was assessed comparing *E.coli* bacteria bioluminescence intensity with LB background noise. As shown on Figure 3B, the background noise of *E.coli* cells (53.14 RLU) was a little superior to LB background noise (48.93 RLU) but not significantly superior. *E.coli* cells have little background noise production. However, this signal is greatly inferior to the one produced by our biosensor in presence of pollutant.

Little leakage in Gaussia luciferase expression.

We investigated the inducibility of the Pu promoter to ensure that the Gaussia luciferase is not constitutively produced in our biosensor. To do so we compared the bioluminescence intensity of bacteria containing our biosensor to the bioluminescence intensity of bacteria transformed with a genetic construction that does not contain the Gaussia luciferase gene (BB12: Pr-RBS-XylR), both in a hermetically sealed tube. The obtained results (Figure 3C) indicate that the Gaussia luciferase gene is not produced in a constitutive manner in the cells. The bioluminescence intensity of our biosensor without toluene injection (158.86 RLU) is a little higher than the bioluminescence intensity of cells transformed with BB12 (53.14 RLU). However, this bioluminescence intensity is significantly lower than the bioluminescence intensity of the cells transformed with our biosensor in presence of toluene (results shown in the next section).

Due to the little LB background noise and leakage in Gaussia luciferase synthesis, obtained bioluminescence intensity results were treated as follows. The background noise (LB+toluene) was subtracted to each sample's bioluminescence intensity. The data was then normalized in function of the negative control.

A



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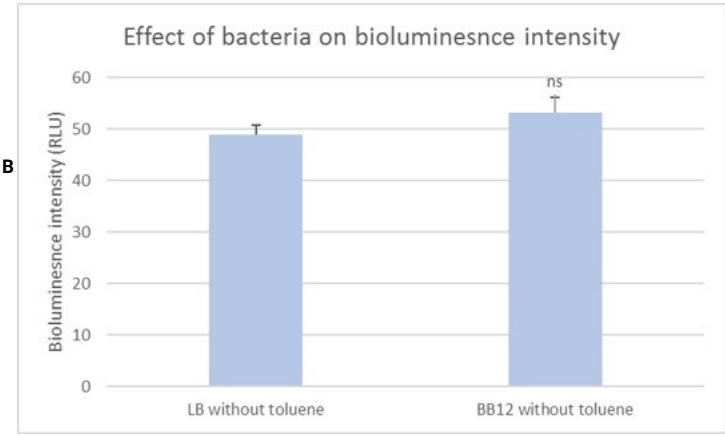

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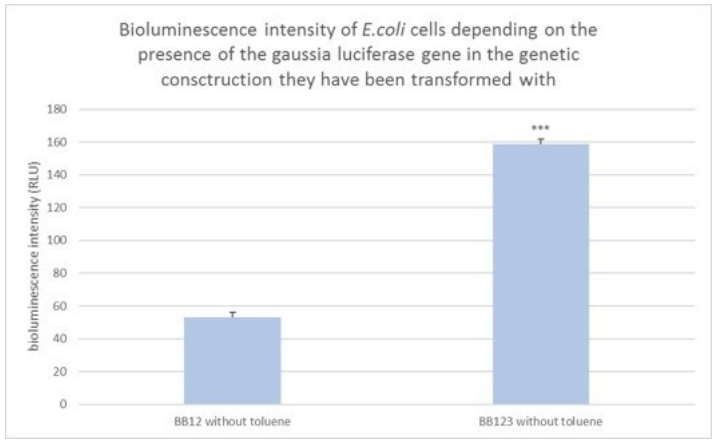
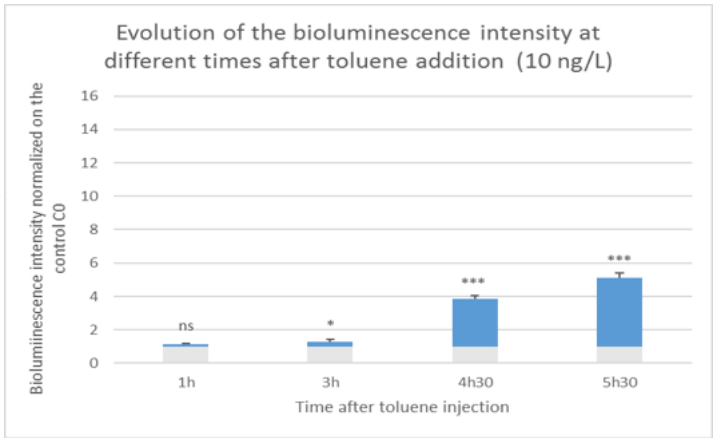


*Figure 3. (A) Bioluminescence intensity expressed in RLU of LB and LB with toluene at 10mg/L. Mann Whitney statistical test was performed to assess the effect of toluene on LB auto-bioluminescence (ns: non-significant). (B) Bioluminescence intensity expressed in RLU of LB and LB + E.coli cells. Mann Whitney statistical test was performed (ns: non-significant). (C) Bioluminescence intensity expressed in RLU of E.coli cells transformed with a construction genetic that contain (BB123) or do not contain (BB12) the Gaussia gene. A little leakage of the Gaussia gene can be seen as the bioluminescence intensity of the sample that contain bacteria transformed with our biosensor is significantly superior to the one that contain bacteria transformed with Pr-RBS-XylR. Mann Whitney statistical test was performed (***: extremely significant: p<0.05).*

A



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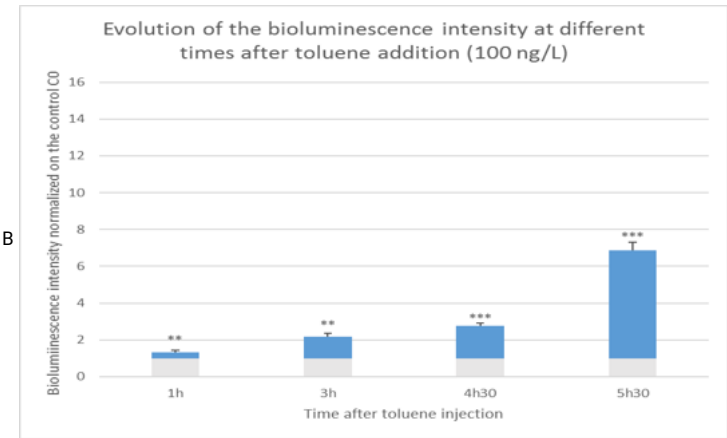

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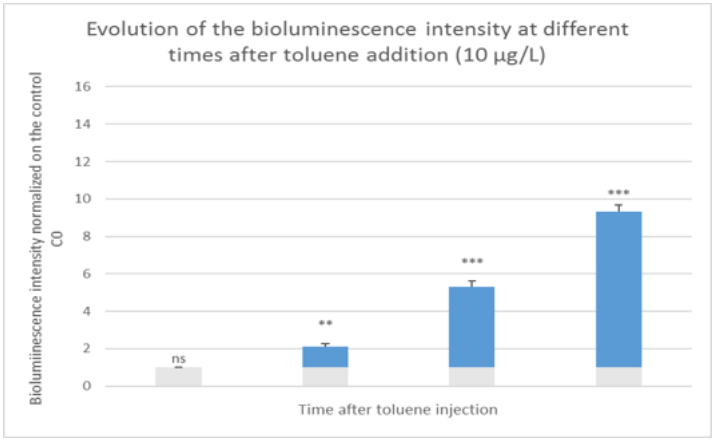

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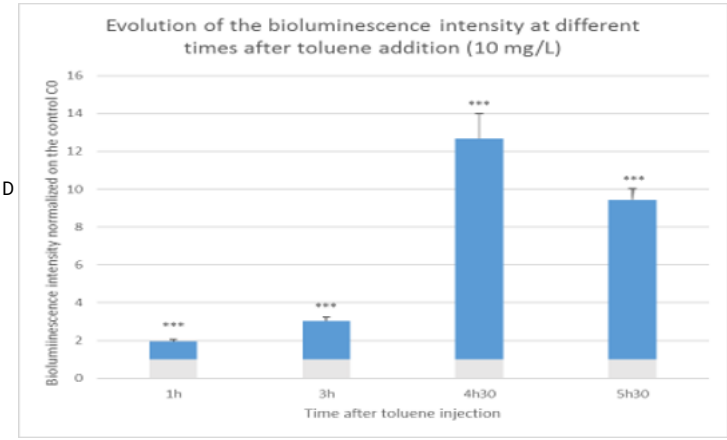

*Figure 4. (A) Evolution of bioluminescence intensity at different times after toluene addition (10ng/L). The bioluminescence intensity of each sample is normalized on the bioluminescence intensity of the corresponding negative control. Mann Whitney statistical test was performed (ns: non-significant; *: p<0.15, significant; **: p<0.1, very significant; ***: p<0.05, extremely significant). (B) Evolution of bioluminescence intensity at different times after toluene addition (100ng/L). The bioluminescence intensity of each sample is normalized on the bioluminescence intensity of the corresponding negative control. Mann Whitney statistical test was performed (ns: non-significant; *: p<0.15, significant; **: p<0.1, very significant; ***: p<0.05, extremely significant).. (C) Evolution of bioluminescence intensity at different times after toluene addition (10µg/L). The bioluminescence intensity of each sample is normalized on the bioluminescence intensity of the corresponding negative control. Mann Whitney statistical test was performed(ns: non-significant; *: p<0.15, significant; **: p<0.1, very significant; ***: p<0.05, extremely significant). (D) Evolution of bioluminescence intensity at different times after toluene addition (10mg/L). The bioluminescence intensity of each sample is normalized on the bioluminescence intensity of the corresponding negative control. Mann Whitney statistical test was performed (ns: non-significant; *: p<0.15, significant; **: p<0.1, very significant; ***: p<0.05, extremely significant).*

Comments

VeroniqueKiermer: You should be careful with the interpretation of p-values. The threshold of 'significance' you are using may be misleading. It's good that you define your statistical test, but it would be even better to show the raw data underlying the bar graphs, to show the spread. What is the n?

Clément Lapierre: This has since been modified, thank you for pointing it out !

f297h834: in table 4, your data suggests that toulene concentration decreases over time due to evaporation. Is this accounted for in your data in this figure? Measurement of toulene injection seems to go up until 5 hours, although it seems like a substantial amount of toulene evaporates by then (~35-40%). Nonetheless, the data in these figures are promising.

Clément Lapierre: This has not been accounted for, as the toluene was added in a sealed tube. There was therefore no evaporation.

Bioluminescence assays results

Investigation of the best measurement time. It was first necessary to determine when, after toluene injection, bioluminescence results were the most relevant. To do so, the bioluminescence intensity of our sample (bacteria transformed with the biosensor within LB medium containing different toluene concentration) was determined several times after toluene addition. The bioluminescence intensity of the negative control was also determined at each time after toluene addition and bioluminescent results for each assay were normalized on the corresponding negative control bioluminescence intensity. This normalization was necessary as we are dealing with several incubation times. This enables to handle the bacteria OD increase within our samples and ensures that an increase in sample's bioluminescence intensity is not correlated with an increase in cells concentration. The graphs Figure 4 represents the bioluminescence intensity of the samples depending on the time after a toluene addition for a fix concentration of injected toluene.

The bioluminescence intensity of all the samples was compared to the bioluminescence intensity of the negative control for each time after toluene injection. Mann Whitney statistics test were realized to determine whether the bioluminescence intensity of the sample was significantly higher than the bioluminescence intensity of the negative control. On the graphics, we can notice that the bioluminescence intensity increase as the time after toluene injection increase. However, for the highest concentration (10 mg/L) (Figure 4D), a decrease in the bioluminescence intensity can be noticed 5h30 after toluene injection. It is due to the accumulation of Gaussia luciferase and the impact of toluene on the cell metabolism and especially protein synthesis. Therefore, it is essential to realize bioluminescence tests at a precise time after sampling. As it is the environmental toluene concentration (less than 20ng/L) that is targeted, it is suitable to wait 5h30 before proceeding to the bioluminescence test. Bacteria were not incubated with toluene for a longer time because of the effect of toluene on *E.coli* cells. We though that this effect will decrease the biosensor sensitivity and precision.

Comments

TrinhNguyen: Hello! It's a very well written article. I could follow all the main ideas easily. I just a a small question for this section. I assume that Gaussia luciferase would accumulate in all other samples with different concentration of toluene. Would it be safe to conclude that accumulation in Gaussia luciferase is part of the reason for reduction in bioluminescence in the highest sample?

Clément Lapierre: Hello ! Thank you for your comment ! No investigation has been made regarding this fact: the accumulation factor was considered canceled by the harmonization of the measurement times: All of the samples have been measured after the same exact period of time following inoculation

Investigation of detectable concentration 5h30 after toluene addition to the cell cultut medium. As it was determined that it is preferable to wait 5h30 before realizing the bioluminescence assay, it was decided to focus on the results obtain for this time after toluene addition in the cells medium. Evolution of the bioluminescence intensity was studied according to the toluene concentration added in the cells medium 5h30 before. Those tests were realized in triplicates. First of all, it was ensured that the OD of the different samples is the same 5h30 after bacteria inoculation in presence of toluene. As shown on Figure 5, the sample's ODs are quite similar going from 1.464 to 1.61.

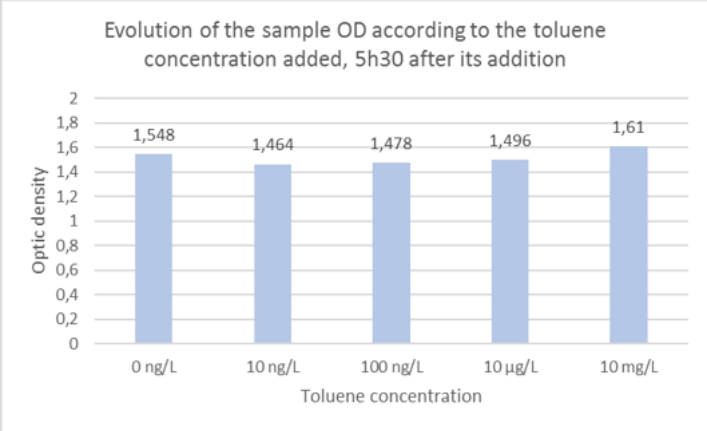

Figure 5. Sample's ODs depending on the toluene concentration added 5h30 before.

The bioluminescence intensity of each sample was then determined and shown depending on the toluene concentration. As we are dealing with extremely different toluene concentration, it was preferable to give our results in function of the logarithm of the toluene concentration. The bioluminescence intensity data in RLU for each replicates (R1, R2, R3) are given in the table below and represented on Figure 6. As shown on the graph, the obtained data differ from one replicates to another due to difference in metabolism, which is normal when working with living organism. However, the curve profile stay is the same: the bioluminescence intensity of the sample increases until a toluene concentration of 10 µg/L, then it reaches a plateau for two replicates and continues to increase for the other replicate. Those results were pooled in order to draw a standard curve and being able to predict toluene concentration in a sample according to the bioluminescence intensity.

Quantification of environmentally relevant toluene concentration. Figure 7 shows the evolution of the bioluminescence intensity depending on the logarithm of toluene concentration. For a toluene concentration inferior to 10µg/L (10,000 ng/L: log (4)) the curve increases steadily, then the curve reach a plateau. This can be due to Gaussia accumulation in the cells. We were able to detect environmentally relevant toluene concentration and as the standard curve is linear for a toluene concentration inferior to 10µg/L, we are able to quantify the toluene concentration of a given sample.

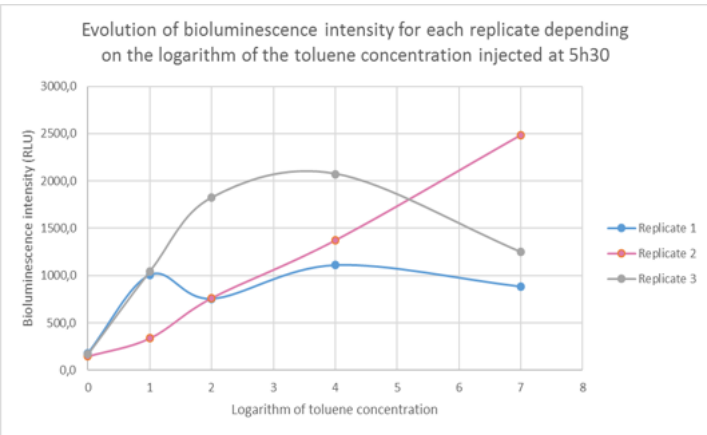

Figure 6. Evolution of bioluminescence intensity for each replicate depending on the logarithm of the toluene concentration injected 5h30 before.

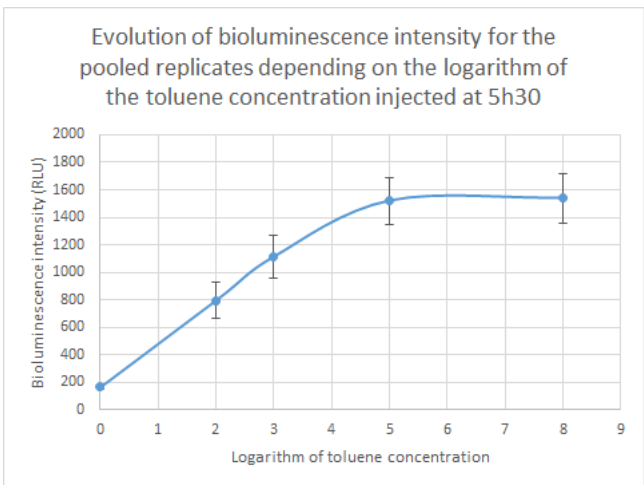

Figure 7. Evolution of bioluminescence intensity for pooled of the replicate depending on the logarithm of the toluene concentration injected 5h30 before. Correlation between the toluene concentration in the cell culture medium and the bioluminescence intensity.

Perspectives

5h30 after toluene injection, the biosensor is able to detect environmentally relevant toluene concentration (10ng/L). This way of pollutant detection is environmentally friendly, as bacteria only required the appropriate molecules in its culture medium to grow and quantify pollutant. In addition, the use of a biosensor, even if it requires an expensive machine (a luminometer) for results analysis, is cheaper compared to others physical chemical existing methods. However, the biosensor is only a prototype and different improvements should be made.

Based on the results present above, we thought about improvement and future experiments that should be realize. First of all, the standard curve drawn is based on few toluene concentrations. This standard curve can be improved by focusing on low toluene concentration and by realizing bioluminescence assays for lower toluene concentrations. The lowest concentration that the biosensor can detect has to be determined while ensuring precise and reproducible results. Moreover, CelloCad software can be used to optimize the genetic circuit.

The variations between the different replicates have to be pointed out for bioluminescence assay. Those variations are due to metabolism variations of living organisms. Several measurements have to be realized for each toluene concentration and the reproducibility of the results have to be assessed on more experiments. Also, the bioluminescence assay protocol has to be optimized and the different parameters of the luminometer have to be tested.

Tests with others pollutants such as the xylene have to be performed. Therefore, the sensibility of the biosensor to those pollutants should be assessed in future experiments. For a convenient reason and as it was not possible to evaporate toluene within the laboratory, bioluminescence assays were only performed using liquid toluene. Future work could consist in the creation of an isolated and hermetic room in which gazeous toluene could be added in order to perform air sampling with the drone.

Comments

ylouis: That's the point ! In fact from the biginning of the work i was wondering why you were trying to compare your experiment in liquid media from environmental conditions in air. Perhaps you should speak of this choice at the beginning as you present this nice work as a proof of concept. Another point have you any example or idea if any other kind of experiment was carried out in liquid media and then transposed to air (and what kind of results you could have with the same hypothesis in terms of concentrations change beetween the 2 media?). What about the exchange layer between air and bacteria ? It will probably be hard to reach the detection of environmental concentration in air ... .

VictorP: First of all, thank you for your comments. During the realization of the project, we have worked on concentration based on result based on toluene concentration in air. Besides, we have tried to find any experiment that allow a comparison between our result obtain in liquid medium and in a gas medium. Nevertheless, it is very difficult to find this because they generally prefer detect pollutants in liquid medium for most of publications that we found. Concerning the exchange layer between air and bacteria, a simple solution is to stir the medium in order to mix the air sample with the liquid medium contained in the sampling tube. We knew that the detection of pollutants in air would be difficult but it was a challenge for us. If you have any other comments, feel free to contact us.

ylouis: Thanks for your answer, i have no more question; your article is well written and presented, perhaps just add some justifications of your choices and the difficulty to work on air sample at the beginning.

VeroniqueKiermer: I agree that you picked a tough challenge! These technical difficulties are understandable and it would be helpful to state them upfront, and explain the choices. You don't speak much about the specificity of the sensor -- are there other chemicals that could be detected with this synthetic construct? That may be an interesting point to discuss.

Clément Lapierre: There is other chemicals, although the XylR mainly focuses on BTEX: no background noise is expected when performing a measurement. As the article focuses on the creation of our Proof of Concept, we did not try any measurement with another pollutant.

f297h834: In your abstract you mention you were able to successfully demonstrate the advantages of whole-cell biosensors with your drone, do you happen to have any data for that?

Clément Lapierre: What we meant in the abstract was that we managed to create the prototype drone that is meant to work with our bionsensor. We couldn't do any testing outside the lab, but the prototype has been proven fonctional. Thank you for your comment !

Acknowledgements

We would like to thank all the people that were here for the project at some point: Damien LASSSALLE and all the other team members, of course, but also our advisors (Alexandre ISMAIL, Samuel JUILLOT, Matthieu DA COSTA, Frank YATES and Estelle MOGENSEN), our schools, our sponsors, the media that spoke about us and the people that were here to support us. Our schools have been extensively supporting our project since the beginning, whether by funding it, granting us access to a lab, allowing some time for our presentations in the planning, lending us the European Experience building, publishing articles about us… The IONIS education group took a big part in our project, and we are very proud that we managed to gather 6 IONIS to work on such a big scale project for the first time in history. Vanessa Proux, director of Sup'Biotech, for her patience, her availability and her constant support when we needed help, or when we wanted to reach some other school directors. All the people from Glowee for their support and help when we needed lab material or emergency advices. Special thanks to Mr. JAISSER and Mr. LECHNER from the INSERM U1138 Team 1 for their answers and availability. They always have been very willing to help us and they have provided us precious support and guidance. Pierre OUGEN, head of the Sup'Biotech Innovative Projects, for lending us a spectrophotometer and a NanoDrop during the project. Davis DU PASQUIER and Andrew TINDALL for their help while analyzing our results. Thomas MARQUES and the Berthold Company for lending us a luminometer, without which our results would have been of a far lesser quality. Thomas LANDRAIN, Cléa BAUVAIS and Marc FOURNIER from the La Paillasse staff, for their help and support.

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Comments

ylouis: complete with Volume 34, Issue 5, p408–419

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Comments

PLOSFeedback: Hi @Clément Lapierre Thanks for your submission to the PLOS iGEM Project. Our editorial team have reviewed your article and have the following feedback: This report describes an interesting concept for a bioluminescence sensor based on E coli to detect the volatile pollutant toluene. The sensor is based on the use of a reporter construct expressing luciferase under the control of the Pu promoter, which is activated by a complex of the XylR protein bound to toluene. The appeal of the new sensor is its portability, allowing it to be deployed precisely at measurement sites via drone. The authors characterize the range of detection of the sensor, the optimal measurement time, and some important elements like the potential toxicity of toluene to the live sensor. The report of the work performed is solid. However, the work as it currently stands is still too preliminary for submission to PLOS ONE. However, the authors would be very welcome to submit their article as an iGEM Report for posting on PLOS Collections (http://blogs.plos.org/collections/). Regardless of where the article is finally submitted, it will be included in the PLOS iGEM 2016 Collection (http://collections.plos.org/igem2016). The authors rightly identify further developments. In addition they may consider characterizing the sensitivity, precision and specificity of the sensor to further the proof of principle.

Clément Lapierre: Hello, Thank you very much for this opportunity. Do you know what we need to do next in order to publish our article as an iGEM Report ? Best regards,

theresa: Hi guys great job! I think your paper is clear and presents your work well. I would suggest making it more reader friendly by moving images to right after they were referenced and also being consistent throughout with labeling of figures. I think your idea for a bioluminescent sensor is really cool I just was wondering if you were able to detect any other chemicals with your biosensor and also why you picked toluene in this experiment?

Clément Lapierre: Hello, thank you for your interest ! We are theoretically able to detect any BTEx molecule, but we worked only with the toluene for safety reasons.