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Research Letter

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Twin-layer biosensor for real-time monitoring of alkane metabolism

Tapio Lehtinen*,†, Ville Santala and Suvi Santala

Tampere University of Technology, Department of Chemistry and Bioengineering, PO Box 541 FIN-33101, Tampere, Finland

*Corresponding author: Tampere University of Technology, Department of Chemistry and Bioengineering, PO Box 541 FIN-33101, Tampere, Finland. Tel: +358 43 211 0739; E-mail: tapio.lehtinen@tut.fi
One sentence summary: A twin-layer biosensor for studying alkane degradation or biosynthesis in *Acinetobacter baylyi* was developed and characterized.
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†Tapio Lehtinen, http://orcid.org/0000-0003-2036-391X

ABSTRACT

Intracellular metabolic sensors can be used for efficient screening and optimization of microbial cell factories. In particular, the sensors are useful in acquiring information about pathway dynamics and bottlenecks in a straightforward manner. Here, we developed a twin-layer biosensor that functions simultaneously at two levels: through transcription factor mediated sensing and enzyme-metabolite interaction, providing insights into the dynamics of alkane metabolism. In addition, the sensor can be used for monitoring either alkane degradation or biosynthesis, depending on the used cellular context. Alkanes are monitored using a fluorescent reporter green fluorescent protein placed under a native alkane-inducible promoter, whereas a bacterial luciferase producing bioluminescence signal enzymatically detects a specific metabolic intermediate in the alkane production/degradation pathway. First, we employed the sensor to investigate the native alkane degradation route in *Acinetobacter baylyi* ADP1. The highest fluorescence and luminescence signals were obtained for dodecane. Second, we constructed a non-native alkane synthesis pathway in *A. baylyi* ADP1, of which the functionality was confirmed with the sensor. The twin-layer approach provides convenient means to study and optimize the kinetics and performance of the heterologous pathway and will facilitate the development of an efficient cell factory.

Keywords: alkane; biosensor; LuxAB; GFP; *Acinetobacter baylyi* ADP1; real-time monitoring

INTRODUCTION

In synthetic biology, biological systems are harnessed for the production of valuable molecules by means of rational design and construction. The typical workflow consists of design, construction, characterization and revision of the synthetic systems. With the advances in computational modeling as well as synthetic DNA and cloning technologies, the phenotypical characterization of the constructed organisms remains the bottleneck for the efficient development of high-performing microbial hosts (Zhang, Jensen and Keasling 2015). This is mainly because the analysis of the metabolic end products and intermediates is laborious and time consuming. In rare exceptions, the positive changes are readily detectable as changes in the phenotype (e.g. colorful product such as lycopene), but usually the metabolites cannot be quantified in a high-throughput manner. Furthermore, for the identification of bottlenecks or imbalances in the metabolic network, the analysis of the end products alone is insufficient and should be complemented with the analysis of key metabolic intermediates.

One solution is to use a suitable biological sensor that is able to respond to changes in the concentration of a specific metabolite and report it with an easily quantifiable signal (Eggeling, Bott and Marienhagen 2015; Zhang, Jensen and Keasling 2015).

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Application of biosensors for the detection and quantitation of selected metabolites allows for high-throughput screening of producer strains (Schallmey *et al.* 2014). In an optimal case, the sensor allows for real-time, non-destructive analysis of key metabolites.

Metabolic sensors can work on different levels; they can report for example changes in promoter activity, protein or RNA expression, or concentration of a specific metabolite. By combining information from different levels, changes in the dynamics of the metabolism can be observed. Information about the pathway dynamics can reveal important aspects that would not be detectable or accessible with end metabolite analysis alone. Moreover, the sensors are often more sensitive and specific compared to conventional analyses methods. In this study, we introduce a biosensing system working on two levels: the system directly detects the presence of molecules of interest, alkanes, by a transcription factor mediated sensing, as well as provides information about the dynamic intermediate compounds, aldehydes, by an enzyme-metabolite interaction. The system can be applied for studying both the alkane degradation and synthesis, depending on the cell context. To highlight the modularity, the system was constructed using a bacterial strain *Acinetobacter baylyi* ADP1, which has not been employed for alkane synthesis before.

Microbial alkane production is a highly interesting option for the replacement of fossil fuels and chemicals (Schirmer *et al.* 2010; Choi and Lee 2013). However, current titers are too low for practical applications. One of the reasons for the low titers is the inefficient flux from acyl-CoA to alkanes through a toxic aldehyde intermediate. Several strategies have been tested in order to improve the production (Andre *et al.* 2013; Wang and Lu 2013), but the work is hindered by the lack of time- and cost-effective methods for the efficient screening of potential genetic modifications and producer strains. Thus, intracellular alkane biosensors could greatly facilitate the development of an efficient alkane-producing microbial factory (Kang and Nielsen 2016).

Various whole-cell sensors for alkane detection have been developed (Sticher *et al.* 1997; Minak-Bernero *et al.* 2004; Reed, Blazeck and Alper 2012; Zhang *et al.* 2012). These sensors are based on the induction of either a luciferase (LuxAB) or a green fluorescent protein (GFP) by an artificially constructed or naturally alkane-responsive promoter. However, those sensors have only been applied in the context of extracellular alkane detection. Recently, Wu *et al.* (2015) developed an alkane sensor for the detection of intracellular alkanes produced by the sensor strain. In the sensor, the alkanes produced by the cells activate the expression of GFP under an artificially constructed alkane-inducible promoter. This was the first demonstration of *in situ* alkane detection within the producer strain using a biosensor. The aforementioned reporters are based on a transcriptional activation of a reporter gene, and consequently show relatively slow responses.

Previously, we developed a simple and robust sensor for extracellular alkanes based on the novel synthetic biology chassis *A. baylyi* ADP1 (Santala, Karp and Santala 2012). This sensor relied on two important aspects of *A. baylyi* metabolism; first, its natural ability for efficient alkane uptake and degradation, and second, the generation of a specific intermediate of the alkane degradation pathway. The intermediate, long-chain aldehyde is a specific substrate for a light-producing bacterial luciferase LuxAB, thus eliminating the need for the addition of external substrate or development of synthetic induction systems.

In the current study, we wanted to improve our LuxAB-based alkane sensor by adding a second sensory level, allowing the detection of alkanes through a reporter transcript output, mediated by a ligand responsive transcription factor. This was achieved by placing a *gfp* gene under a native alkane-inducible promoter (Ratajczak, Geissdorfer and Hillen 1998b). As a result, the twin sensor simultaneously detects alkanes and alkane degradation/biosynthesis intermediates (long-chain aldehydes), offering insights into the dynamics of the metabolism. The twin sensor was employed for monitoring the natural alkane degradation route as well as a heterologous alkane biosynthesis pathway.

MATERIALS AND METHODS

Bacterial strains

The wild-type *Acinetobacter baylyi* ADP1 (Deutsche Sammlung von Mikroorganismen und Zellkulturen, DSM 24193) was used in the study. For plasmid construction and maintenance, *Escherichia coli* XL1-Blue (Stratagene, California, USA) was used.

Genetic modifications

The molecular work was carried out using established methods. The reagents and primers were purchased from ThermoFisher Scientific (Massachusetts, USA) and used according to the manufacturer's instructions. Antibiotics were used in concentrations of 25 μg ml^{-1} for chloramphenicol and 50 μg ml^{-1} for kanamycin and spectinomycin, when appropriate. Transformation of *A. baylyi* was carried out as described previously (Metzgar *et al.* 2004). All genome modifications were confirmed with PCR and sequencing.

The genetic cassette for the introduction of *gfp* to the genome of *A. baylyi* under the alkane-inducible promoter was assembled sequentially by restriction cloning using a previously described integration cassette *iluxAB_Cm*^{r}/pAK400c as the backbone (Santala *et al.* 2011a). The construct contains a *gfp* gene and a kanamycin resistance marker flanked by the upstream and downstream regions of *AlkM*. The upstream flanking region contained the alkane-inducible promoter. The 3′ and 5′ flanking regions were PCR-amplified from *A. baylyi* genome with primers tl01 & tl02 and tl09 & tl10, respectively (Table 1). A gene encoding superfolder GFP (sfGFP) was amplified from the BioBrick collection (BBa_I746915) with primers tl03 and tl04 and the kanamycin resistance marker from plasmid pBAV1K-T5-gfp (Bryksin and Matsumura 2010) with primers tl07 and tl08. *Acinetobacter baylyi* was transformed with the cassette and strains characterized based on their ability for n-alkane utilization. For the external alkane detection, a strain capable of alkane degradation was used (indicating that the integration of the cassette did not knock out the *alkM* gene). For internal alkane production, a strain with a functional *alkM* knockout was used in order to prevent the degradation of the synthesized alkanes.

For the introduction of the *luxAB* genes into the genome, a previously described cassette was employed (Santala *et al.* 2011b). This cassette is designed to knock out the ACIAD3383-3381 genes, thereby removing the endogenous fatty acyl-CoA reductase and pyruvate dehydrogenase activities. The *luxAB* genes were cut from the *iluxAB_Cm*^{r}/pAK400c plasmid using *NdeI* and *XhoI* restriction enzymes and cloned to the cassette.

For the alkane-producing strain, a construct was prepared where *aar (Synechococcus elongatus PCC7942_orf 1594)* and *ado (S. elongatus PCC7942_orf 1593)* genes are under T5/lac promoter in

Table 1. Primers used in the study.

Name	Sequence 5′ → 3′	Description
tl01	taagcgggtaccgtcgtaccatcctgtaatgtgaaatgag	AlkM 5′ flanking forward, KpnI
tl02	tacaggcaattgcatagtgaatcctttcttgttatcctc	AlkM 5′ flanking reverse, MfeI
tl03	gactaacaattgcgtaaaggcgaagagc	sfGFP forward, MfeI
tl04	aatgcctctagatcatcatttgtacagttcatccatacc	sfGFP reverse, XbaI
tl07	gcgagcctcgagaaggaagctaaaatggctaaaatgagaatatcaccgg	kanR forward, XhoI
tl08	aggaaactgcagctaaaacaattcatccagtaaaatataatattttattttctcc	kanR reverse, PstI
tl09	aacggccctaggtcagaccttattttaaaagagag	AlkM 3′ flanking forward, AvrII
tl10	attgccggcccccgaggccaaagcaggccgtatctgtaatg	AlkM 3′ flanking reverse, SfiI
tl24	cgtggaattggtaccctagatgttatttcttcaaaattatgg	pIM1463 1/2 forward, KpnI
tl25	gatgatctttctctagaagcggccgcgaattcgcatgctctagtaagcttctcc	pIM1463 1/2 reverse, XbaI
tl26	agggagtctagaactagtagcggccgctgcagaaaggagaagcttactagc	pIM1463 2/2 forward, XbaI
tl27	tgcagcggcccccgaggccaagcttacctgaaagccaat	pIM1463 2/2 reverse, SfiI

an integrative cassette. The integrative region of the plasmid pIM1463 (Murin *et al.* 2012) was PCR amplified in two parts with primers (tl24, tl25, tl26 and tl27) designed in such a way that the *gusA* gene becomes replaced by a BioBrick accepting cloning site (EcoRI-XbaI-SpeI-PstI). *Aar* and *ado* genes were codon-optimized and purchased from Genscript (New Jersey, USA) with appropriate restriction sites and ribosomal binding sites and placed under the promoter using standard methods. The construct contained flanking sites to facilitate the integration into a prophage region in the genome of *A. baylyi* ADP1.

Luminescent and fluorescent measurements

For the induction with external alkanes, the cells were grown overnight in the LB medium (5 g l^{-1} NaCl, 5 g l^{-1} yeast extract, 10 g l^{-1} tryptone) supplemented with 0.4% glucose. The measurement was done in 96-well plate, with 200 μl of the overnight culture and ∼40 mM of alkane added. Between the measurements, the plate was incubated in 30°C and shaking. Optical density (Multiscan Ascent, Thermo Labsystems, Finland; wavelength 540 nm), fluorescence (Fluoroskan Ascent FL, Thermo Labsystems, Finland) using the wavelengths 485 nm (half band width (HBW) 14±2 nm) for excitation and 538 nm (HBW 25±3 nm) for emission, and luminescence (Wallac 1420 Victor2, Perkin Elmer, Finland; 1 s measurement time) measurements were performed every 15 min.

For the measurement of internally produced alkanes, the cells were grown on Luria-Agar (LA) (5 g l^{-1} NaCl, 5 g l^{-1} yeast extract, 10 g l^{-1} tryptone, 15 g l^{-1} Agar-agar) on 48-well plate inside Xenogen *In Vitro* Imaging System (IVIS® Lumina, Caliper Life Sciences, USA) at 30°C. Luminescence and fluorescence (excitation: 445–490 nm, emission: 515–575 nm) signals were measured every hour. To investigate the possible overlap of the emission wavelength of the luciferase with the excitation profile of the GFP, the fluorescence emission was also measured without the excitation (the excitation filter was closed). Any 'autofluorescence' obtained in this setting would result from the luciferase exciting the GFP, but the signal was found to be negligible compared to the actual fluorescent signal. The relative fluorescence signal was calculated by dividing the fluorescence of the sensor strain with the fluorescence of wild-type *A. baylyi* ADP1 grown in the same conditions. For confirming the alkane production, GC-MS analysis was carried out (Supplementary File 1, Supporting Information).

For the visualization of the fluorescence from single colonies, the sensor cells were grown overnight in LB, diluted and plated on LA plates with 100 μM isopropyl β-D-1-thiogalactopyranoside (IPTG, ThermoFisher Scientific). From the control plates, the IPTG was omitted. The plates were incubated in 30°C for 24 h and visualized with a Safe Imager Blue Light Transilluminator (ThermoFisher Scientific).

RESULTS AND DISCUSSION

To detect the presence of alkanes by the reporter system, a native alkane-responsive promoter (P_{alkM}) of *Acinetobacter baylyi* was exploited in the construction of the sensor. The promoter is activated in the presence of alkanes by a transcriptional activator AlkR (Ratajczak, Geissdorfer and Hillen 1998b). For monitoring the levels of specific intermediates, long-chain aldehydes, a constitutively expressed bacterial luciferase LuxAB was employed. The bacterial luciferase uses aldehydes as a substrate in the reaction producing a fatty acid molecule and measurable light signal (bioluminescence).

The twin sensor for monitoring of the alkane degradation in *A. baylyi* (designated as EAS for external alkane sensor) is presented in Fig. 1 (left). The sensor was created as follows: a synthetic gene cassette was constructed consisting of the P_{alkM} promoter (sequence immediately upstream of *alkM*), a *gfp* gene and a kanamycin resistance marker. The cassette was flanked by homologous regions facilitating its integration to the genome by homologous recombination (Elliott and Neidle 2011). LuxAB was introduced using another genetic cassette, containing the *lux A* and *B* genes under constitutive promoter and flanking regions, facilitating the integration to the genetic locus, replacing the genes ACIAD3381–ACIAD3383 and thus eliminating the native aldehyde synthesis (Santala *et al.* 2011a).

Because *luxAB* is constitutively expressed, the detection of aldehydes by luminescence is not dependent on transcription or the presence of an inducer. Thus, the luminescence response is instant and dynamic. In contrast, the production of fluorescence signal is a slow process and the detected signal is cumulative in nature, due to the working mechanism of the reporter including induction event and protein expression as well as the relatively high stability of GFP.

The response of the twin sensor to external n-alkanes of various chain lengths is presented in Fig. 2. The alkanes induce transcription from the alkane-responsive promoter, which can be observed as the increase in the fluorescence signal. As the alkane degradation pathway activates, the formation of long-chain aldehydes is seen as the increase in the luminescence signal. Induction of the luminescence and fluorescence signals

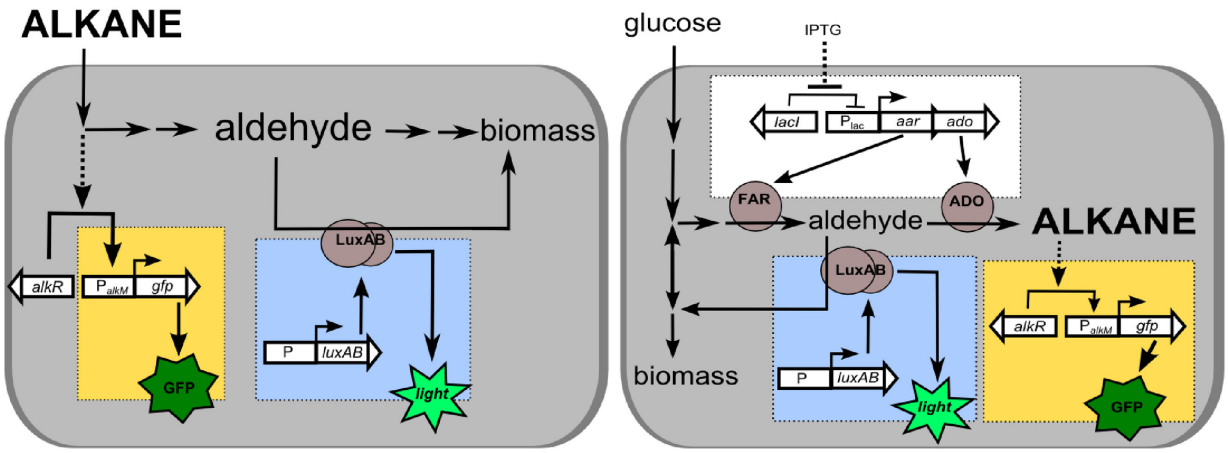

Figure 1. Twin-layer sensors for monitoring alkane degradation and synthesis. The sensors can be used for the monitoring of alkane degradation (left) or biosynthesis (right). The twin sensor components encompass a constitutively expressed *luxAB* encoding for bacterial luciferase, which produces bioluminescence upon interaction with the aldehyde intermediate (blue module), and an alkane inducible reporter consisting of the transcription factor AlkR and the cognate promoter P_{alkM} regulating the expression of GFP (yellow module). For alkane biosynthesis (right), a module containing the activities of a fatty acyl-CoA reductase (FAR) and an aldehyde-deformylating oxygenase (ADO) under inducible promoter system (*lacI*-P*lac*) was added.

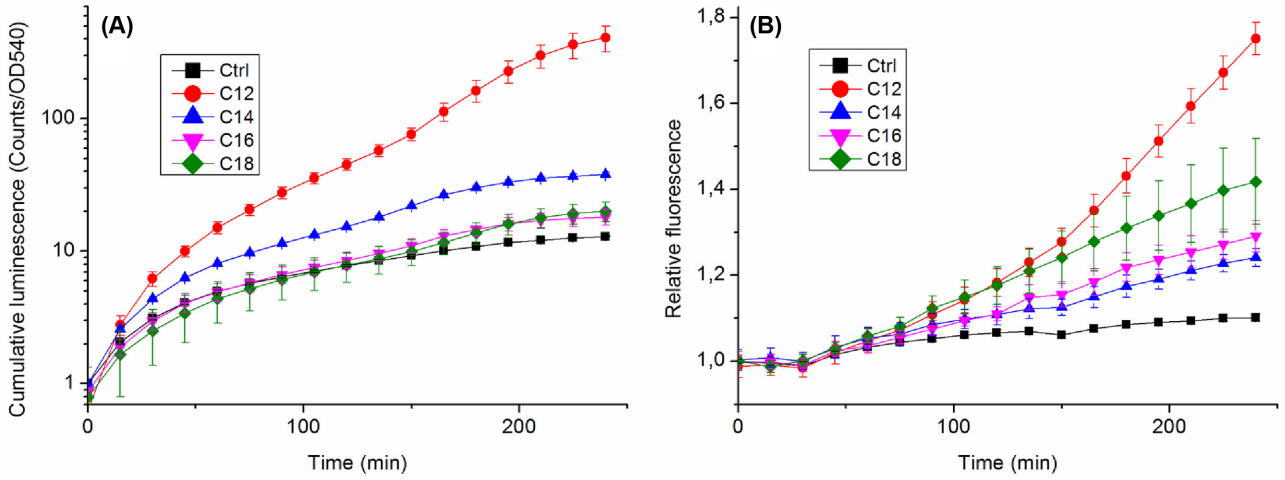

Figure 2. Luminescent and fluorescent responses of sensor cells to external alkanes. The EAS sensor cells (expressing LuxAB constitutively and GFP under an alkane-inducible promoter) were cultivated in LB overnight, divided into 96 well plate and aliphatic alkanes with carbon chain lengths of 12–18 added (final concentration 40 mM). Glucose (40 mM) was added to the control samples. The luminescent (**A**) and fluorescent (**B**) responses were monitored every 15 min. Relative fluorescence is defined as the fold change of the fluorescence signal divided by the OD_{540} of the sample. The luminescence signal is presented relative to the optical density. The optical densities are provided in Table S1 (Supporting Information). The signals are presented as average of three replicates. Error bars represent standard deviation.

behaves differently with varying alkane chain lengths. This can be attributed to the different chain-length preferences of LuxAB and the AlkR-P_{alkM} induction system. Among the tested alkanes, dodecane (C12) yields the highest signals in both luminescence and fluorescence, being consistent with our earlier results (Santala, Karp and Santala 2012). In the previous studies (Ratajczak, Geissdorfer and Hillen 1998b; Zhang *et al.* 2012), octadecane has been observed as the strongest inducer of AlkM expression. The differences can be due to different experimental settings or reporter systems in the genetic level. For example, the differences in solubility between the alkanes with different chain lengths might cause variation between the experimental setups. With octadecane (C18), the fluorescence induction is stronger than the luminescence, whereas with tetradecane the luminescence induction is stronger. For the luminescence signal, the differences in the induction can be partially explained with the substrate specificity of the luciferase; for example, tetradecanal (C14) is preferred over longer carbon chains by LuxAB (Colepicolo *et al.* 1989), explaining its relatively higher activity. The variations in the luminescence and fluorescence signal ratios in response to alkanes of different chain lengths further emphasize the importance of monitoring metabolic pathways at multiple levels.

Apart from the detection of the external alkanes, we wanted to use the sensor for studying the alkane biosynthesis. *Acinetobacter baylyi* exhibits relatively high tolerance towards hydrocarbon metabolites and naturally accumulates large amounts of acyl-CoA and aldehyde-derived hydrocarbons as storage compounds, thus making it an appealing host for microbial alkane production (Santala *et al.* 2011b; Santala, Karp and Santala 2012). Although *A. baylyi* has been previously employed as a robust host for genetic investigations, metabolic engineering and

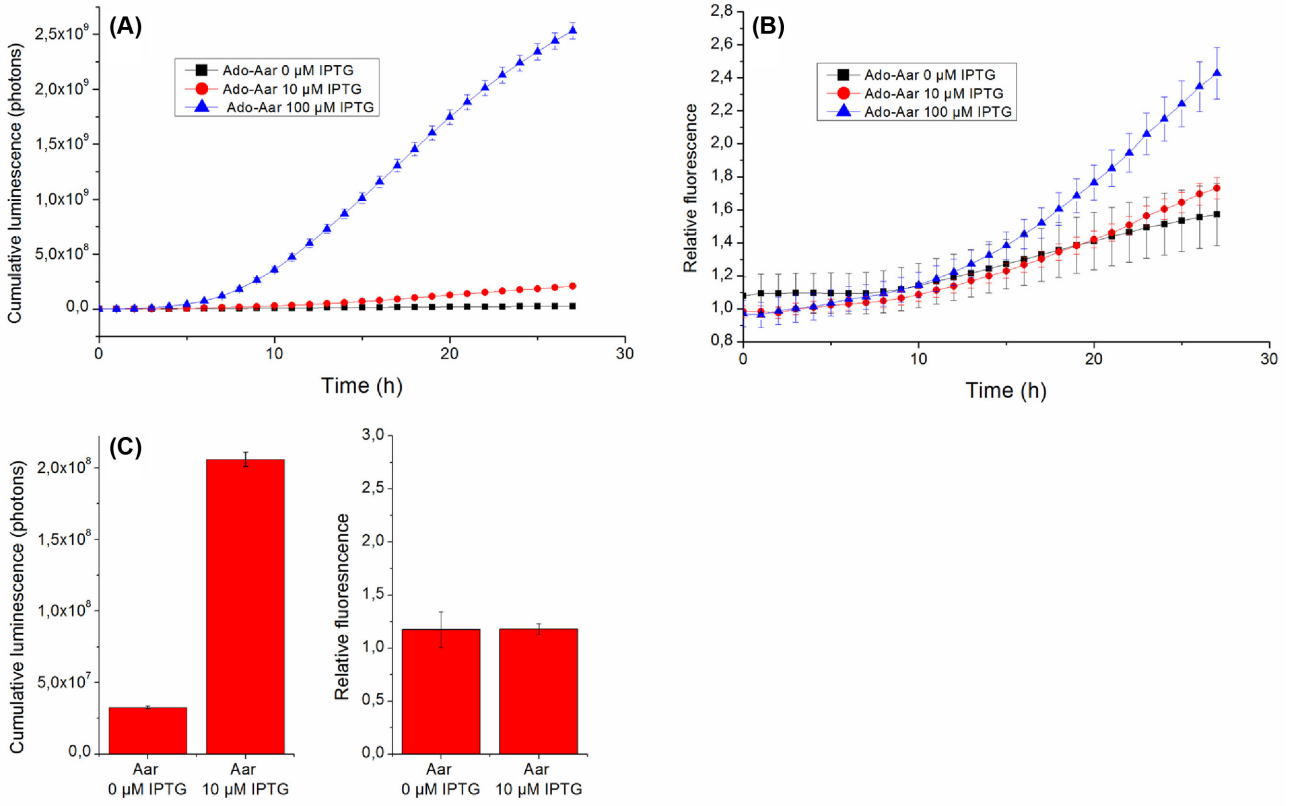

Figure 3. Monitoring the biosynthesis of alkanes. The IAS sensor cells (ADP1ΔACIAD3381-3383::*luxAB*, Δ*alkM*::*gfp*, harboring the alkane synthesis element with *ado* and *aar* under the IPTG-inducible lac/T5 promoter) were grown on LA plates in the presence of different IPTG concentrations. The constitutively expressed LuxAB measures the production of the aldehyde intermediate (**A**), while the expression of GFP is dependent on the activation of the alkane-inducible promoter P_{alkM} by alkanes (**B**). For control, responses of sensor cells expressing only Aar and not Ado were measured (**C**). The signals are presented as average of three replicates. Error bars represent standard deviation. Relative fluorescence was calculated by dividing the fluorescence signal of the sensor cells with the fluorescence signal of wild-type *A. baylyi* ADP1 cells grown in the same conditions.

synthetic biology (de Berardinis *et al.* 2009; Murin *et al.* 2012; Santala, Karp and Santala 2014; Santala *et al.* 2014; Kannisto *et al.* 2015), further research is required to investigate its potential for industrial use.

For the detection of internally produced alkanes, a different version of the twin sensor was used and a genetic module harboring the elements for alkane biosynthesis was added to the sensor strain (Fig. 1, right). In this sensor strain, alkane degradation is prevented by a knockout of *alkM*, which encodes for alkane hydroxylase and is essential for alkane degradation in *A. baylyi* (Ratajczak, Geissdorfer and Hillen 1998a). Alkane biosynthesis has been achieved in *Escherichia coli* by the expression of two cyanobacterial genes, *aar* and *ado* (Schirmer *et al.* 2010), which encode enzymes with the activities of fatty acyl-ACP reductase and aldehyde-deformylating oxygenase (ADO), respectively. We applied the two widely used components to investigate alkane biosynthesis in *A. baylyi*. The sensor strain was transformed with a construct containing *ado* and *aar* under a lac/T5 promoter and homologous regions to facilitate the integration of the cassette to the genome. To our knowledge, this is the first time when a naturally alkane degrading strain is engineered to produce alkanes. In this twin sensor/producer strain, designated as IAS (for internal alkane sensor), the same reporter elements LuxAB and GFP allow the monitoring of long-chain aldehydes produced by Aar and alkanes produced by Ado, respectively (Fig. 1, right). Similarly to EAS strain, the gene cassette eliminating ACIAD3381-3383 was used for the insertion of *luxAB*. The ACIAD3383 gene encodes for a native fatty acyl-CoA reductase of *A. baylyi*. It was desirable to remove this activity in order to eliminate the endogenous fatty aldehyde production. The ACIAD3381 encodes for a pyruvate dehydrogenase, a deletion of which has been associated with potentially higher wax ester production (Santala *et al.* 2011b). Thus, it seemed possible that its deletion could also be beneficial for alkane production. ACIAD3382 encodes for a homocysteine synthase, and its deletion is neutral in terms of growth and lipid production (Santala *et al.* 2011b); the ACIAD3382 gene was deleted for practical reasons to allow the deletion of ACIAD3383 and ACIAD3381 with a single cassette. It is also notable that LuxAB consumes aldehyde molecules in the light-producing reaction. However, the fatty acid molecules produced in the reaction are recycled by the cells, which somewhat alleviates the possible effect on alkane production. Furthermore, we have previously used a similar detection system for the analysis of wax ester production pathway, and have found that the luciferase does not have a significant influence on the production (Santala *et al.* 2011a). Thus, the overall effect of the LuxAB activity on the production of alkanes can be considered negligible.

In order to study the alkane biosynthesis, Aar and Ado were expressed under an IPTG-inducible promoter with different concentrations of the inducer. The luminescence and fluorescence signals were measured in real time during the cell growth.

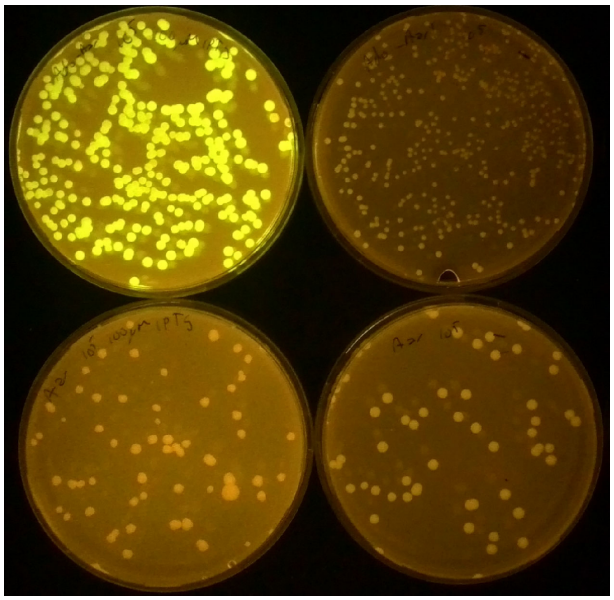

Figure 4. Detection of fluorescence from single colonies on agar plate. The alkane-producing sensor cells were plated from liquid culture on agar plate with 100 μM IPTG (top left) or without IPTG (top right) and incubated for 24 h. The bottom row is the sensor cells with only Aar and no Ado, with and without IPTG. The plates are illuminated with a blue light transilluminator.

In the presence of the inducer, a response was seen in the luminescence and fluorescence signals, indicating the production of alkanes via the aldehyde intermediate (Fig. 3). In both signals, a positive correlation was seen with the IPTG concentration and the signal strength, indicating a dynamic behavior of the sensor. The alkane production of the Ado-Aar expressing strain was also confirmed with GC-MS analysis (Supplementary File 1). A moderate induction (10 μM IPTG) of Aar alone (for control) resulted in luminescence but not fluorescence production, as expected. However, with higher inducer concentrations the expression of Aar resulted in inconsistent cell growth, potentially caused by the toxic effect of long-chain aldehydes.

Biosensors can speed up the development of efficient producer strains by allowing for high-throughput screening of different genetic modifications. Currently, practical technologies allowing the selection of single cells in a high-throughput manner are limited to fluorescence-activated cell sorting, which requires special instrumentation. Thus, it would be highly convenient to be able to screen the best phenotypes directly from colonies grown on plates. To assess the applicability of our twin sensor for high-throughput screening, individual cells were plated on agar plates and the signal strength was evaluated from single colonies. The fluorescence was readily detected from single colonies grown in the presence of the inducer (Fig. 4), suggesting that the sensor would be amenable to high-throughput screening directly on plates.

A major challenge in metabolic engineering is to balance the metabolic pathway in a way that the flux is directed to the desired product and accumulation of potentially toxic intermediates is minimized (Jones, Toparlak and Koffas 2015). One option is to screen for various natural or modified enzymes and their expression levels to find a combination that results in maximal product formation. In the alkane biosynthesis pathway, the fatty aldehyde production must be carefully balanced in order to provide ADO with sufficient substrate load while avoiding the accumulation of the fatty aldehydes, which can cause toxic or inhibitory effects (Cao *et al.* 2016). The twin configuration of the sensor enables the detection of both the intermediate and the end product, which allows more targeted optimization of the production pathway than a sensor working on a single level. The optimal production strain should show high fluorescence-to-luminescence ratio, indicating that aldehydes are effectively converted to alkanes and do not accumulate in the cells. It is well known that ADOs are relatively slow enzymes, with k_{cat} values $\sim$1 min^{-1} (Marsh and Waugh 2013). Various strategies have been attempted to increase the turnover rate, including the removal of inhibition (Andre *et al.* 2013) and improving the substrate availability by scaffolding (Sachdeva *et al.* 2014). Another approach to improving the alkane synthesis could involve protein engineering of ADOs. The sensor presented here could serve as an efficient tool for screening the variants.

In this study, we developed and characterized a novel twin biosensor for the analysis of alkane metabolism. The sensor can be applied for the detection of both external and internally produced alkanes in real time. The twin configuration of the sensor makes it possible to simultaneously detect signals from two different levels, offering insights into the dynamics of the metabolism. Furthermore, this is the first report describing alkane production in the promising synthetic biology chassis *A. baylyi*. The presented sensor system holds potential for speeding up the metabolic engineering work aiming for efficient alkane production.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSLE* online.

AUTHOR CONTRIBUTIONS

TL, SS and VS designed the study. TL performed the molecular and microbiological work, analyzed the data and wrote the manuscript. SS and VS supervised and coordinated the study. All authors approved the final version of the manuscript.

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Conflict of interest. None declared.

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