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A Yeast-Based Biosensor for Screening of Short- and Medium-Chain Fatty Acid Production

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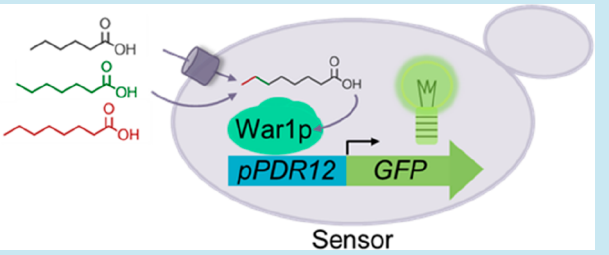
Supporting Information

ABSTRACT: Short- and medium-chain fatty acids (SMCFA) are important platform chemicals currently produced from non-sustainable resources. The engineering of microbial cells to produce SMCFA, however, lacks high-throughput methods to screen for best performing cells. Here, we present the development of a whole-cell biosensor for easy and rapid detection of SMCFA. The biosensor is based on a multicopy yeast plasmid containing the SMCFA-responsive *PDR12* promoter coupled to GFP as the reporter gene. The sensor detected hexanoic, heptanoic and octanoic acid over a linear range up to 2, 1.5, and 0.75 mM, respectively, but did not show a linear response to decanoic and dodecanoic acid. We validated the functionality of the biosensor with culture supernatants of a previously engineered *Saccharomyces cerevisiae* octanoic acid producer strain and derivatives thereof. The biosensor signal correlated strongly with the octanoic acid concentrations as determined by gas chromatography. Thus, this biosensor enables the high-throughput screening of SMCFA producers and has the potential to drastically speed up the engineering of diverse SMCFA producing cell factories.



KEYWORDS: *biosensor, high-throughput screening, short-chain fatty acids, medium-chain fatty acids, octanoic acid, PDR12*

Short- and medium-chain fatty acids (SMCFA) and their derivatives have a wide range of industrial applications such as pharmaceuticals, antimicrobials or biofuels.[1] Rising concerns about nonsustainable production methods, such as deforestation and competition with the food industry, makes the fermentative production from renewable resources by microbial cell factories increasingly attractive. In recent years, several reports about the successful engineering of strains for the production of SMCFA have been published, but yields, titers and productivities remain low.[2] One of the reasons for this is the lack of high-throughput screening methods for randomly mutated or rationally engineered strains to identify highly producing cells, as the common screening methods currently applied are mostly based on low-throughput gas (GC) or liquid chromatography (LC).[3]

The use of biosensors for screening has become a popular alternative to chromatography and can substantially accelerate the optimization of production strains.[4] Most developed biosensor systems rely on the expression of sensing elements, *e.g.*, genes encoding extracellular receptors, under the control of an inducible promoter, and are coupled with an easily detectable output such as fluorescence, luminescence, colorimetry or growth rate.[4]

To our knowledge only one whole-cell SMCFA biosensor was developed so far, which was also based on this principle.[5] It made use of heterologous G-protein coupled receptors (GPCRs) in combination with the yeast mating pathway for signaling and a reporter construct inducing GFP expression upon exposure to octanoic acid (C_8 FA) and decanoic acid (C_{10} FA). Although this GPCR-based sensor system has the advantage of being amenable to the sensing of other compounds by exchanging the receptor unit,[6] it has some drawbacks including complicated strain construction (several gene deletions and insertions), the need for functional expression of heterologous plasma membrane localized receptors as well as a low linear range for C_8 FA detection. Most importantly, the functionality of this sensor could not be verified in a mixture with other secreted acids, *i.e.*, myristic and palmitic acid (C_{14} and C_{16} FA), which was partially attributed to the toxicity of FA on cell growth.[5] However, functionality of a sensor in a mixture of different acids, as found in culture supernatants, would be essential for practical application.

Due to these limitations, we assumed that a promoter-based biosensor for SMCFA detection could be a more promising approach. There are several examples of transcription factor/promoter-based biosensors developed for the detection of environmental pollutants but not so many for the screening of mutant libraries to identify best performing cells.[7−11] A prerequisite for the use of such systems as biosensors is the

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identification of a substrate-specific transcription factor and its respective promoter.[12] The *PDR12* promoter appeared as a suitable candidate for the sensor system that we envisioned, as it is rapidly activated by only one transcription factor[13,14] and responds to a narrow spectrum of substrates.[13,15−17] *PDR12* encodes an ATP-binding cassette transporter and is under control of the Zn_2Cys_6 transcription factor War1p.[13,14] War1p constitutively binds to a weak acid response element (WARE) in the *PDR12* promoter. Upon weak acid stress, War1p is phosphorylated and undergoes conformational changes leading to its activation and thereby *PDR12* expression.[14,18,19] *PDR12* has been shown so far to be induced by multiple substrates, *i.e.*, propanoic, butanoic, pentanoic, hexanoic and heptanoic acids (C_3−C_7 FA), C_8 FA,[15,17,20] sorbate and benzoate[13] but not by acetate,[16] succinate, citrate[17] or organic alcohols.[15] The role of Pdr12p in response to C_3−C_8 FA has been demonstrated with a Δ*pdr12* mutant that was hypersensitive and the activation of a *PDR12*-lacZ reporter construct in response to these FA.[15,17] Furthermore, a microarray analysis revealed the activation of *PDR12* expression upon exposure of yeast cells to C_8 FA.[20] In addition to these characteristics, the *PDR12* promoter has a low constitutive activity in the absence of stress,[14] but upon the appropriate signal exposure, Pdr12p becomes one of the most abundant plasma membrane proteins.[21]

These previous findings led us to the assumption that GFP expression driven by the *PDR12* promoter could be suitable as a biosensor for SMCFA. We further sought to develop it as a two-cell sensor system in which SMCFA sensing is decoupled from SMCFA production for several reasons. First, with increasing titers, the producer microbe supernatant can simply be diluted to stay in the linear range of the sensor, which also helps to evade detrimental effects of SMCFA on yeast growth at higher concentrations.[22] A one-cell sensor system could lead to false-negatives due to a saturation of the sensor signal at concentrations exceeding the linear range. Second, the producer microbe can be further engineered or modified without affecting the sensor function. Finally, we want to select for cells producing high extracellular titers. In a one-cell system, an intracellular sensor will only measure the cytosolic amount of produced SMCFA, which can lead, for example, to the identification of transport-deficient mutants that accumulate SMCFA intracellularly instead of producing higher titers.

Previously, we engineered an *S. cerevisiae* strain producing mainly C_8 FA with titers of up to 245 mg/L (1.7 mM) and minor amounts of C_6, C_{10} and C_{12} FA.[23] Interested in improving this strain for higher titers, we aimed at developing a biosensor assay for rapid detection of SMCFA in culture supernatants of this strain and mutants thereof.

RESULTS AND DISCUSSION

Design of a *PDR12* Promoter-Based Biosensor for SMCFA. We sought to develop a two-cell sensor system in which SMCFA sensing is decoupled from SMCFA production as schematically illustrated in Figure 1. The biosensor was designed by fusing the *PDR12* promoter (corresponding to 1168 bp upstream of the *PDR12* coding sequence)[15] to the open reading frame of enhanced GFP, namely Envy,[24] and inserted into a multicopy plasmid that was transformed into *S. cerevisiae* CEN.PK113−11C. The sensor's functionality relies on the uptake of exogenous SMCFA, such as C_8 FA, by the yeast cells.[25] At physiological pH, C_8 FA are taken up *via* active transport, whereas at acidic pH the undissociated acid form can enter the cell by passive diffusion.[22,26] Once taken up by the cell, the C_8 FA activates War1p, leading to transcription from the *PDR12* promoter. We found that GFP expression is activated only in the presence of the inducing signal (here: C_8 FA); in the absence of signal, we observed only low constitutive fluorescence (Figure S1). To prove the functionality of the biosensor in culture supernatants, we used a previously constructed *S. cerevisiae* strain (RPY21/FAS^{R1834K}) that produces mainly C_8 FA, which is secreted from or diffuses out of the cells.[25]

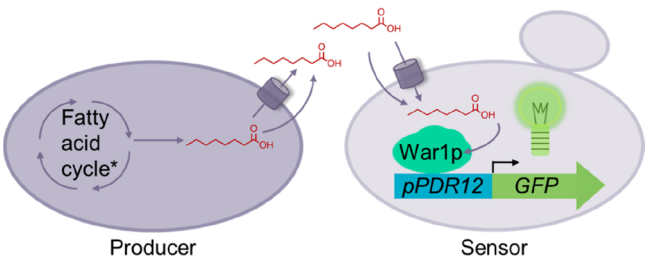

Figure 1. Short- and medium-chain fatty acid detection by a whole-cell biosensor. A producer microbe secretes short- and/or medium-chain fatty acids (SMCFA), such as octanoic acid shown here, which are produced through a modified fatty acid cycle (*). The culture supernatant is added to the yeast biosensor strain, which takes up the SMCFA *via* diffusion or active transport. The biosensor consists of the *PDR12* promoter (*pPDR12*) coupled to an enhanced version of green fluorescent protein (*GFP*). The transcription factor War1p, which is constitutively bound to *pPDR12*, undergoes phosphorylation and conformational changes upon signal exposure and initiates the expression of GFP.

Characterization and Verification of Functionality of the Biosensor. We analyzed the biosensor response to C_8 FA concentrations between 0 and 1 mM for an initial validation in defined medium (SCD). We observed a correlation of the RFI (relative fluorescence intensity) of the biosensor to C_8 FA over the entire concentration range and a linear response between 0.01 and 0.75 mM (Figure 2A), which is a strong improvement over a previously published GPCR-based C_8 FA sensor (0.019−0.25 mM linear range).[5] The highest intensity of the signal after activation showed a nearly 10-fold increase after 2 h of incubation with C_8 FA, displaying a high dynamic range (Figure 2A, Figure S2). To make sure that the activation of the biosensor is specific, we tested a control strain with a plasmid expressing GFP under control of the *MET25* promoter, which is methionine repressible. In this control strain we observed no C_8 FA concentration-dependent induction of GFP expression in SCD medium without methionine (Figure 2A).

Industrial microbial fermentations commonly use complex medium rather than defined medium. Therefore, we next tested the biosensor in a complex medium, *i.e.*, YPD. Again, we observed a correlation of the RFI to the tested C_8 FA concentrations (0−3 mM) and an identical linear range between 0.01 and 0.75 mM (Figure 2B, Figure S3). While the dynamic range of the sensor was lower than in SCD medium even after twice the incubation time (Figure S4), this can be attributed to the higher background fluorescence of YPD. Nevertheless, the maximum increase in the intensity of the signal after activation was more than tripled after 4 h of incubation. Even though there is a discrepancy in previous reports about the *PDR12* induction by C_8 FA,[15,17,20] we could clearly show that the *PDR12* promoter's C_8 FA-dependent response allows it to be used as a biosensor. To our knowledge, this is the first SMCFA biosensor shown to function reliably in

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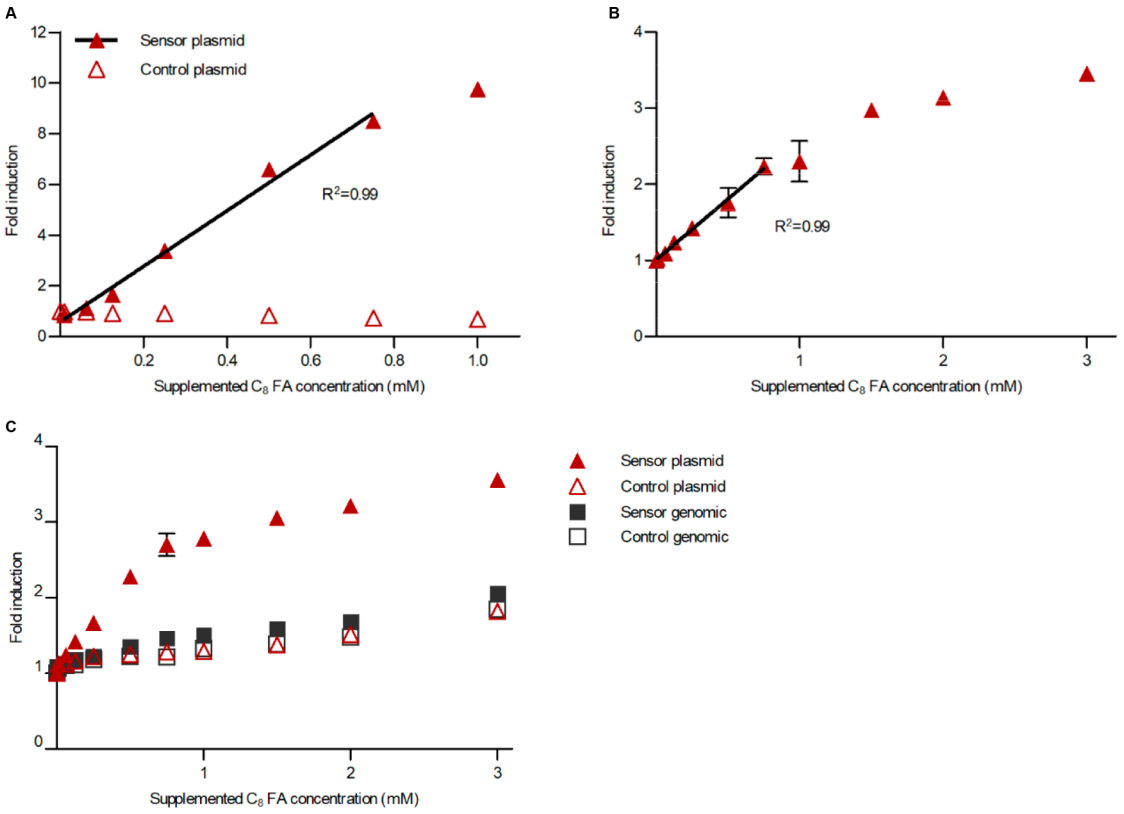

Figure 2. C_8 fatty acid-dependent response of plasmid-based and genomically integrated biosensors. Response and linear range of the plasmid-based biosensor after 2 h incubation with C_8 fatty acids (FA) in SCD (A) and after 4 h in YPD medium (B). Error bars represent two technical replicates. Experiments were conducted in three biological replicates with comparable results (Figure S2, Figure S3). (C) Response of the genomically integrated biosensor after 4 h incubation with C_8 FA in YPD. Sensor plasmid: CEN.PK113−11C + p426pPDR12-GFP. Control plasmid: CEN.PK113−11C + p426pMET25-GFP. Sensor genomic: LBY27. Control genomic: CEN.PK113−11C. For fold induction, fluorescence intensities (FI) were divided by optical densities (OD_{600}) and normalized to FI/OD_{600} values of samples without C_8 FA.

complex medium. This is of particular importance since the highest SMCFA titers produced in yeast so far were reached in complex medium.[23,27,28]

To avoid possible stability problems by using a multicopy plasmid-based biosensor, we integrated the sensor construct into the *S. cerevisiae* CEN.PK113−11C genome resulting in strain LBY27. This sensor strain, however, only showed a barely detectable increase in the intensity of the signal upon C_8 FA addition in comparison to the control CEN.PK113−11C strain (Figure 2C). Therefore, a multicopy plasmid seems to be necessary for a good dynamic range, and we continued to work with the plasmid-based biosensor.

The Biosensor Also Responds to C_6 and C_7 but Not C_{10} or C_{12} FA. It was shown in previous work that *PDR12* expression is influenced by several weak acids.[13,15−17] As none of these compounds—except other SMCFA—are usually produced by *S. cerevisiae*, we reasoned that they should not influence the biosensor's ability to detect SMCFA in *S. cerevisiae* culture supernatants.

The previously engineered *S. cerevisiae* strain RPY21/FAS^{R1834K} mainly produces C_8 FA and in minor amounts C_6, C_{10} and C_{12} FA. To test whether the biosensor also detects these byproducts, we analyzed its response upon addition of C_6, C_{10} and C_{12} FA to YPD. As depicted in Figure 3, the RFI of the biosensor correlates to C_6 FA levels between 0 and 3 mM, and the biosensor showed a linear response between 0.01 and 2 mM in all replicates (Figure S3). C_{10} FA seems to be very toxic to the cells, as growth is already strongly decreased at the

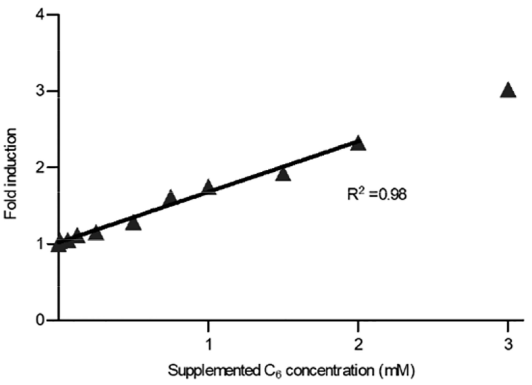

Figure 3. C_6 fatty acid-dependent response of the biosensor. Response and linear range of the biosensor after 4 h incubation with C_6 fatty acids (FA) in YPD medium. Error bars represent two technical replicates. Experiments were conducted in three biological replicates with comparable results (Figure S3). For fold induction, fluorescence intensities (FI) were divided by optical densities (OD_{600}) and normalized to FI/OD_{600} values of samples without FA.

lower concentrations, which leads to false high inductions when dividing FI by OD_{600} values (Figure S3). This is in accordance with previous findings about high toxicity of C_{10} FA.[22] Overall, we cannot exclude a very slight response of the biosensor to C_{10} FA at low concentrations (0.01−0.25 mM), which would be in accordance to a previous report suggesting a discrete role of Pdr12p in C_{10} FA response.[17] Nevertheless, we

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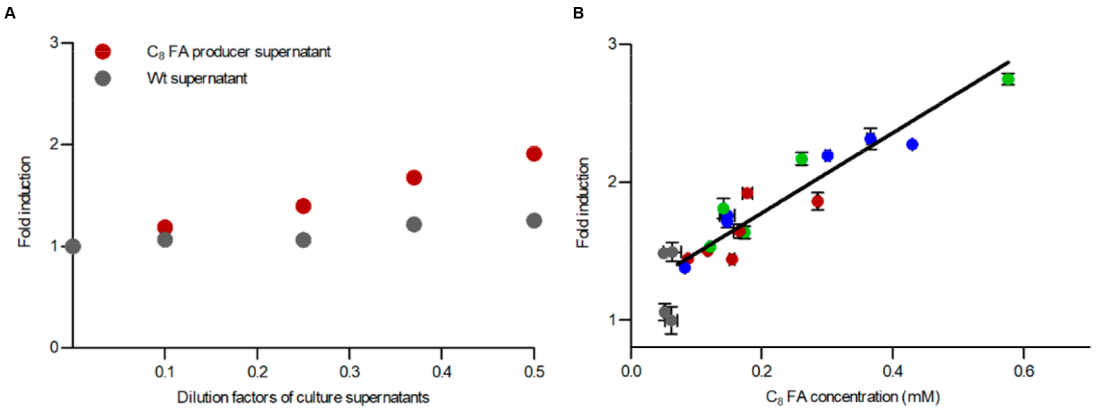

Figure 4. Biosensor response to C_8 fatty acids in *S. cerevisiae* culture supernatants and correlation to GC measurement. (A) Response of the biosensor to differently diluted culture supernatants of a C_8 fatty acid (FA) producer (RPY21/FAS^{R1834K}/pRS42H) and a wildtype strain (Wt, CEN.PK113−7D). (B) Linear correlation of the fold induction of biosensor signal in 0.5 dilutions of culture supernatants with GC measurements of the same supernatants. Strains: CEN.PK113−7D (gray), RPY21/FAS^{R1834K}/pRS42H (red), RPY21/FAS^{R1834K}/LBV17 (blue), RPY21/FAS^{R1834K}/LBV20 (green).

could not observe a linear response range of the biosensor to C_{10} FA in all three biological replicates.

For C_{12} FA, we found a concentration-dependent decrease in fluorescence over the entire concentration range tested and an increase in the OD_{600} at C_{12} FA concentrations between 0.25 and 1 mM (Figure S5). We also observed this when adding C_{12} FA to plain medium not containing the biosensor strain (data not shown). Therefore, this is probably due to incomplete solubility and light scattering by C_{12} FA micelles. In addition, we also tested the biosensor response to the odd chain C_7 FA and observed a linear response between 0.01 and 1.5 mM (Figure S6). This is in accordance to previous studies reporting that *PDR12* is induced by C_7 FA.[15,17]

In summary, we show that the biosensor responds to C_6, C_7 and C_8 FA in YPD medium over linear ranges from 0.01 up to 2, 1.5, and 0.75 mM, respectively, whereas it does not show a linear response to C_{10} FA and no concentration-dependent response at all to C_{12} FA.

Detection of C_8 FA by the Biosensor in *S. cerevisiae* Culture Supernatants. To validate that the biosensor can discriminate between different SMCFA concentrations in culture supernatants, we first conducted a pretest. We cultivated a C_8 FA producer strain (RPY21/FAS^{R1834K}/pRS42H) and a wildtype strain (CEN.PK113−7D) in buffered YPD (with hygromycin if applicable) for 72 h and added the culture supernatants in different dilutions to the biosensor, and in parallel we measured the C_8 FA content by GC. As shown in Figure 4A, the lowest dilution (0.5) of the C_8 FA producer supernatant led to a 1.9-fold increase in the intensity of the signal after 4 h of incubation. Furthermore, we observed a constant increase in signal intensity with decreasing dilution factors confirming the C_8 FA concentration-dependent activation of the biosensor in *S. cerevisiae* culture supernatants. The supernatant of the wildtype culture also led to a slight increase in signal intensity of the biosensor, which can be attributed to minor amounts of C_8 FA that it produced naturally. As determined by GC, the wildtype strain produced 0.02 mM C_8 FA, whereas RPY21/FAS^{R1834K}/pRS42H produced 0.28 mM C_8 FA.

To obtain supernatants with a wide range of C_8 FA concentrations we not only tested the original RPY21/FAS^{R1834K} strain but also two derivatives thereof. We transformed the strain with one of two plasmids containing overexpressed genes or the control plasmid (pRS42H), respectively. One plasmid harbored an overexpression construct of *TPO1* (LBV17). The transporter Tpo1p seems to be involved in the resistance to C_8 FA[20] and therefore has the potential to decrease C_8 FA toxicity. The second construct encoded the acetyl-CoA carboxylase *ACC1* and harbored mutations leading to amino acid exchanges of two serine residues to alanine (*ACC1*^{S659AS1157A}; LBV20). These two mutations impair the deactivation of Acc1p *via* phosphorylation.[29−31] A similarly modified Acc1p (*ACC1*^{S1157A}) was shown to have a positive effect on yeast resistance to C_8 FA,[32] and therefore we reasoned that it could also influence C_8 FA product titers of RPY21/FAS^{R1834K}. We cultivated RPY21/FAS^{R1834K} with overexpression or control plasmids as well as a wildtype strain (CEN.PK113−7D) in two independent experiments in two to three replicates and obtained a wide range of C_8 FA titers between 0.05 and 0.58 mM as measured by GC. We added the culture supernatants to the biosensor (corresponding to a 0.5 dilution) and measured the RFI. As shown in Figure 4B, the RFI of the biosensor clearly correlates with the C_8 FA concentrations measured by GC. A linear correlation could be observed for concentrations of produced C_8 FA from 0.08 mM up to the highest concentration tested, 0.58 mM. We additionally tested the supernatants in a dilution of 0.25 with the biosensor and received—despite lower fold inductions—an identical linear range (Figure S7A). In all samples, the C_6 FA content was below 6% of the total SMCFA titers and did not alter the linear correlation of the biosensor response to C_8 FA concentrations (Figure S7B).

This is the first report of a whole-cell promoter-based biosensor for the detection of SMCFA in culture supernatants. In contrast to previously generated sensor systems,[5,6,8−10] the here presented biosensor solely consists of an *S. cerevisiae* strain transformed with a multicopy plasmid containing the *PDR12* promoter coupled to GFP without further strain engineering, fine-tuning or expression of additional components needed. We demonstrate that it linearly responds to C_8 FA in defined and complex medium as well as to C_6 and C_7 FA in complex medium. Furthermore, the biosensor responds to C_8 FA concentrations from 0.08 up to at least 0.58 mM in *S. cerevisiae* culture supernatants and therefore in a mixture of other secreted FA. The biosensor response showed a clear correlation of RFI to C_8 FA values determined by GC and

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therefore can be used to compare SMCFA titers of different culture supernatants. On the basis of these results, we envision two possible applications. It can be used as a screening system of microbial producer strain libraries of one specific SMCFA, as shown for the C_8 FA producing strain here, or to screen for increased overall SMCFA titers. For the latter, GC measurements can be used to determine the exact SMCFA composition of the culture supernatants once a biosensor-aided screening resulted in several good performing candidate strains.

The currently produced amounts of C_6 FA by yeast vary from 0.17 to 0.62 mM[23,28,33] and fall within the linear range of the biosensor. On the other hand, C_8 FA titers produced by yeast vary from 0.45 to 1.7 mM[23,28,33] and therefore partly exceed the linear range of the biosensor. However, we can simply dilute culture supernatants before adding them to the biosensor to retain a linear response. The biosensor also has a wide linear range for C_7 FA detection and could be a valuable tool to screen a C_7 FA-producer library; however, yeast has not been engineered to produce C_7 FA so far.

For future use, a more stable, plasmid-independent sensor strain might be beneficial. This could be achieved by a multicopy integration of the sensor into the genome. Thereby, the low dynamic range of the sensor response as seen with the single genomic integration might be increased. The dynamic range of the sensor could be further optimized by the genomic replacement of the native *WAR1* promoter with the *PDR12* promoter.[11] Assuming low "leaky" expression levels of *WAR1*, such a positive-feedback loop would accelerate *WAR1* expression in the presence of inducing molecules. These higher War1p levels would then result in a higher expression of *PDR12* promoter-coupled GFP, possibly leading to higher dynamic ranges of the sensor. To improve the linear range of the biosensor, the saturation of the sensor signal would have to be shifted toward higher FA concentrations. One possibility to achieve this could be to decrease the binding affinity of War1p to the WARE site in the *PDR12* promoter. This might be accomplished either by mutating *WAR1* or the WARE site. Alternatively, War1p could be mutated to exhibit a lower binding affinity for its inducers; however, it is known neither if the inducing molecules directly bind War1p nor at which molecular site.[14,18,19] In order to try any of these approaches, detailed War1p structural data would be needed.

Nevertheless, the here presented biosensor has the appropriate linear and dynamic ranges to use it for high-throughput screening of yeast SMCFA production. Needing only 4 h of incubation of sample supernatants with the biosensor in multiwell format, hundreds of strains can be rapidly screened to select the best performing strains. For future use, we envision a broader application spectrum of the biosensor as its use is not restricted *per se* to *S. cerevisiae* or even yeast SMCFA producers. The biosensor's SMCFA response certainly will have to be confirmed in the supernatants of other microbes, *i.e.*, other yeasts or bacteria, as different species also produce different metabolites that could influence the biosensor's response to SMCFA in other culture supernatants. Overall, this biosensor represents a valuable and ready-to-use system, which fills a need to rapidly improve microbial SMCFA production.

METHODS

Strains and Plasmid Construction. Yeast strains and plasmids used throughout this study are listed in Table S1. The *PDR12* promoter, *TPO1* and $ACC1^{S659AS1157A}$ as well as promoters (*pPGK1*, *pPYK1*) and endogenous terminators (*tTPO1*, *tACC1*) were amplified from CEN.PK113−11C genomic DNA with primers containing the respective overhangs for cloning *via* homologous recombination (oligonucleotides are listed in Table S2). The *PDR12* promoter was integrated into the *Sac*I site of p426pMET25-GFP, thereby replacing *pMET25*. The genomic insertion of *pPDR12-GFP* was performed *via* CRISPR/Cas9 as described previously.[34] The CRISPR/Cas9 plasmid was amplified in two PCR fragments and transformed for *in vivo* assembly. The insertion fragment was amplified from plasmid p426pPDR12-GFP. Yeast transformations were performed according to Gietz and Schiestl[35] or for RPY21 *via* an adapted procedure.[23] To generate RPY21/FAS^{R1834K}, RPY21 was transformed with a plasmid carrying the wildtype version of *FAS2* and another plasmid carrying *FAS1* with mutations leading to an amino acid replacement (R1834K), thereby redirecting the FA production from long chain FA to SMCFA.[23] Cells were streaked out on selective YPD (1% yeast extract, 2% peptone, both produced by BD, Difco Laboratories, Sparks, USA; 2% D-glucose, purchased from Roth, Karlsruhe, Germany) containing hygromycin or G418 (200 μg/mL) to select for *hphNT1* or *kanMX* or on selective SCD medium[36] lacking leucine, tryptophan, uracil and/or histidine (± LWUH) to select for *LEU2*, *URA3* or *HIS3* markers, respectively. Electrocompetent *E. coli* DH10*β* (Gibco BRL, Gaithersburg, MD) was used for subcloning according to standard procedures, and transformants were selected on lysogeny broth (LB) agar plates[37] supplemented with 100 μg/mL ampicillin.

Cultures for Fatty Acid Production. C_8 FA producer and control (wildtype) strains were grown as previously described[23] with minor adjustments. For precultures, several colonies of the strains were picked and combined in 20 mL YPD with 100 mM potassium phosphate buffer (pH 6.5). After shaking at 180 r.p.m. at 30 °C overnight, the main culture was inoculated to an OD_{600} of 0.1 in 30 mL buffered YPD medium and cultured in 300 mL shake flasks under the same conditions. After 72 h, the cultures were harvested by centrifugation and 20 mL of the supernatant was used for FA extraction (see below) whereas remaining supernatant was used for the biosensor assay.

Fatty Acid Extraction and Quantification *via* GC. FA extraction and GC analysis were performed as described previously.[25] For each culture, two 10 mL aliquots from the same culture supernatant were separately processed and measured by GC. The standard deviation (SD) between the two measurements from the same culture was for all samples below 2 mg/L.

Cultures for the Biosensor Assay. For the biosensor assay, strains (CEN.PK113−11C/p426pMET25-GFP or p426pPDR12-GFP) were grown overnight with 180 r.p.m. shaking at 30 °C in SCD-L-W-U and under addition of repressive concentrations of methionine (2 mM) for the strain harboring plasmid p426pMET25-GFP. Precultured cells were used to inoculate 20 mL of fresh SCD-L-W-U (+2 mM methionine) to an OD_{600} of 0.1 and grown for 4−6 h until an OD_{600} of about 0.4. Cells were centrifuged and resuspended in fresh YPD or SCD-L-W-U without methionine and transferred into black 96-well plates with clear flat bottom (ref. 655097, Greiner Bio-one, Frickenhausen, Germany) with 100 μL per well. To keep conditions sterile, well plates were sealed with a gas permeable sealing membrane (Breathe-Easy, Diversified

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Biotech, Dedham, MA, USA). For the primary verification of the sensor, 90 μL of fresh media was added together with 10 μL of 70% v/v ethanol (control) or 10 μL of differently diluted fatty acids (in 70% v/v ethanol) to always reach a final volume of 200 μL/well and have the same ethanol concentrations in all wells. C_6, C_7, C_8, C_{10} and C_{12} FA (Sigma-Aldrich) were diluted in 70% v/v ethanol in concentrations between 0.2 and 60 mM to reach final dilutions of 0.01 to 3 mM in the wells. For the biosensor test with culture supernatants, 100 μL of culture supernatant (for cultivation conditions see above) was added to 100 μL of the biosensor strain. Experiments were performed in biological triplicates with technical duplicates.

Biosensor Measurements. Directly after pipetting of all components into the 96-well plate, it was incubated at 30 °C and 600 r.p.m. shaking in a CLARIOstar plate reader (BMG Labtech, Ortenberg, Germany). Measurements of OD_{600} and FI with excitation at 470 ± 15 nm and emission at 515 ± 20 nm were taken every 30 min over several hours. To normalize for different cell numbers in wells, the FI value of each well was divided by the respective OD_{600} value. For calculating the fold induction, these FI/OD_{600} values were divided for the different FA concentrations by the FI/OD_{600} values of the "0 mM" controls (without FA). For calculation of the fold induction response to the culture supernatants, FI/OD_{600} values of the wells with supernatant and biosensor were divided by FI/OD_{600} values only containing biosensor. Values are shown as mean ± the standard deviation (SD).

Data analyses including the calculation of linear regression and R^2 values were performed using the software Prism 5 (GraphPad, USA).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssynbio.8b00309.

Supporting figures and tables (PDF)

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Author Contributions

LB, EB and MO conceived the study. ASR and JPM provided ideas and constructive input. LB performed all experiments. LB, EB and MO analyzed the data and wrote the paper. All authors read and approved the manuscript.

Notes

The authors declare the following competing financial interest(s): EB is inventor of EP patent application No. 15 162 192.7 filed on April 1, 2015, and of EP patent application No. 15 174 342.4 filed on June 26, 2015, by Goethe-University Frankfurt, concerning short-chain acyl-CoA producing FAS variants. There are no other competing interests.

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■ ABBREVIATIONS

SMCFA, short- and medium-chain fatty acid(s); FA, fatty acid(s); C_6, C_7, C_8, C_{10}, C_{12} FA, hexanoic, heptanoic, octanoic, decanoic, dodecanoic acid(s); FAS, fatty acid synthase; PDR12, plasma membrane ATP-binding cassette transporter; TPO1, polyamine transporter of the major facilitator superfamily; ACC1, acetyl-CoA carboxylase; GFP, green fluorescent protein; GC, gas chromatography; OD_{600}, optical density at λ = 600 nm; FI, fluorescence intensity; RFI, relative fluorescence intensity.

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