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# $O_2$-requiring molecular reporters of gene expression for anaerobic microorganisms

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ABSTRACT

Many genetic reporter systems require molecular oxygen; therefore, the use of reporter genes to study molecular mechanisms in anaerobic microorganisms has been hampered by the lack of convenient reporting systems. We describe reporter gene whole cell-based biosensor systems based on luciferase genes and the associated oxygen-requiring enzymes. By using two different oxygen-dependent reporters, insect and bacterial luciferases, and two bacterial hosts, Gram (+) *Bifidobacterium longum* and Gram (-) *Escherichia coli*, we show that the enzymes can be used in gene expression studies of anaerobic bacteria. *E. coli,* a facultative anaerobe, was grown both in aerobic and anaerobic conditions with an arabinose-inducible expression system. We show that a short treatment time of few minutes in ambient atmosphere is sufficient to detect light emission from living cells that is directly proportional to the number of cells and to the inducer concentration. The induction levels were the same in both the aerobically and anaerobically cultured cells. Similar results were obtained in the case of *B. longum* cultured in anaerobic conditions.

## 1. Introduction

Anaerobic microbes are an important class of organisms because they can be used in a vast number of biotechnological applications, and these microbes have been used since ancient times in the production of food and feed, with wine and beverages being the most well-known products. Upon the origin of life on Earth approximately 3.9 billion years ago (Rizzotti, 2009), the conditions were anaerobic, and the first living organisms were heterotrophic. The abundance of sub-seafloor microbes is expected to be $35.5{\cdot}10^{29}$ cells, comprising 55–86% of Earth's prokaryotic biomass and 27–33% of Earth's living biomass, and most of these cells are anaerobic, methanogenic bacteria or archaea, which dominate in deep sea sediments (Whitman et al., 1998); therefore, they present a vast, nearly unknown reservoir of organisms that have not yet been studied at the gene expression level. Furthermore, anaerobic microorganisms have an enormous market potential in various biotechnological applications, such as anaerobic digestion in sewage treatment systems, bioenergy production ($H_2$), alcohols and methane bioprocesses, production of fermented food products and in metabolic engineering in cosmetics, prebiotics and medicines. Therefore, it is strongly justified to identify convenient and simple methods for studying gene expression in both Gram (+) and Gram (-) anaerobic microorganisms, and also fungi and yeasts.

Research with anaerobic organisms has lagged behind that with aerobic microorganisms, partly because there are too few powerful molecular tools for efficiently studying the intracellular mechanisms in physiology and biochemistry at the molecular level. Reporter genes are tools that are used in place of each target regulon in cases where gene expression and its regulation are studied (Daunert et al., 2000; Elad et al., 2008). The gene encoding ß-galactosidase has been the most widely used reporter in anaerobic research because its product can be easily measured by a simple colour reaction (Feustel et al., 2004). Green fluorescent protein (GFP) and its colour variants have been shown to be unsuitable reporters in anaerobic bacteria because their correct chromophore formation requires aerobic conditions (Hansen et al., 2001; Tsien, 1998). Drepper et al. (2007) showed that blue-light photoreceptors from *Bacillus subtilis* and *Pseudomonas aeruginosa* contain light-oxygen-voltage-sensing domains that can be engineered to work as fluorescent reporters for gene expression analysis under both aerobic and anaerobic conditions. Bioluminescent proteins require oxygen as a substrate in their light-emitting reaction. Reporter genes supporting bioluminescent or fluorescent detection technologies have several benefits over other approaches whose activities are detected by spectrophotometry, such as ß-galactosidase (Feustel et al., 2004) and alkaline phosphatase (Edwards et al., 2015), or radioactivity (chloramphenicol acetyltransferase), namely, high sensitivity and real-time

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detection. Most of these approaches, with the exception of luciferases, require cell disruption as an extra step in activity measurements. In principle, luciferase reporters have the highest sensitivity because biological samples do not produce light of their own (except for light-emitting organisms), given that the measurement chamber is a black, light-tight box that blocks ambient light. The use fluorescent reporters suffers from cellular autofluorescence that is triggered upon excitation, thus lowering the sensitivity. Both bacterial and insect luciferases (Michelini et al., 2008) are widely used as molecular reporter systems, but their use in anaerobic applications is believed to be restricted by the requirement for oxygen in the light-emitting reaction.

There are few reports on the use of luciferase-based reporters in anaerobic microorganisms. However, there are no in-depth analyses of how they function. For instance, the first paper by Phillips-Jones (1993) described the use of the *Vibrio fischeri luxAB* genes for monitoring gene expression in anaerobically cultured *Clostridium perfringens*. Not until ten years later were the *luxAB* genes used to monitor the effects of antimicrobial agents against nonreplicating, anaerobically grown *Mycobacterium tuberculosis* (Cho et al., 2007). In a decade-old report, fusions of the mercury resistance operon (*mer*) with a promoterless *luxCDABE* operon from *Vibrio fischeri* (Selifonova et al., 1993) were used to study the effect of intracellular pH on the accumulation of trace concentrations in *Escherichia coli* in anaerobic conditions (Golding et al., 2008). In that study, as in the previous examples, the measurement of light emission from the luciferase reporters was measured by shaking the samples in aerobic conditions after growth in anaerobic conditions, and the experimental setups were not shown in detail. It was found that only the β-galactosidase reporter gene worked properly in a tumour hypoxia model when monitored with in vivo imaging (Cecic et al., 2007). This result shows that it is important to provide molecular oxygen to the cells expressing reporter gene products that require oxygen for catalysis.

The light emission reaction by bacterial luciferases has the following formula:

$$FMNH2 + O_2 + RCHO \xrightarrow{\text{luciferase}} FMN + RCOOH + H_2O + h\nu\ (490\ \text{nm})$$

where RCHO is a long-chain aldehyde and RCOOH is the corresponding fatty acid. Light-emission can be obtained from cells containing the structural genes of the bacterial luciferase operon, *luxCDABE*, without any external additives.

The reaction catalysed by insect luciferases has the following formula:

$$ATP + O_2 + D{-}luciferin \xrightarrow{\text{luciferase}} AMP + PP_i + CO_2 + oxyluciferin + h\nu\ (560\ \text{nm})$$

Light-emission can be obtained from cells that are supplied with external D-luciferin.

Both types of luciferase reporter proteins require molecular oxygen for catalysis. We report here that by applying a short recovery step under aerobic atmosphere prior to bioluminescence measurements, bacterial and insect luciferases (biosensing elements) can be used as molecular reporters in anaerobically cultured *Escherichia coli* and *Bifidobacterium longum* model organisms. We also show that gene expression closely parallels the dose-response using induction of the arabinose-inducible promoter in both aerobic and anaerobic conditions.

## 2. Materials and methods

### 2.1. Construction of Sak-Lux plasmid for Escherichia coli expression

The construction of the arabinose-inducible, bacterial luciferase operon-containing plasmid is shown in the Supplementary Material.

### 2.2. Construction and cultivation of bifidobacteria harbouring the shuttle plasmid expressing the click beetle luciferase gene

Electrocompetent *Bifidobacterium longum* cells were prepared as described earlier (Guglielmetti et al., 2007). The human intestinal anaerobic bacterium *B. longum* NCC2705 was transformed by electroporation with a shuttle vector (pGBL8b) containing the insect luciferase gene from a click beetle (*Pyrophorus plagiophthalamus*) under the control of a strong phage T5 promoter. The bioluminescence reporter vector pGBL8b was constructed earlier (Guglielmetti et al., 2008). Electroporation was carried out with a MicroPulser Electroporator (Bio-Rad, Milano, Italy) set at 12.5 kV/cm and employing a 2-mm cuvette (the time constants obtained were between 3.9 and 4.2 ms). This recombinant bifidobacterial strain was cultivated in anaerobic conditions at 37 °C in MRS medium (Difco Laboratories Inc., Detroit, MI) supplemented with 0.05% cysteine-HCl and 10 μg/ml of chloramphenicol. Anaerobic conditions were created by the use of an anaerobic glove box (80% $N_2$, 10% $CO_2$, 10% $H_2$). The resulting clones displayed a bioluminescent phenotype. Light emission was studied in relation to anaerobic conditions.

### 2.3. Aerobic and anaerobic induction experiments for bacterial and insect luciferase reporter systems

pSak-Lux plasmid-containing *E. coli* XL1 was inoculated in 5 ml of L-broth with ampicillin (100 μg/ml) for overnight (O/N) incubation. O/N cells were inoculated into anaerobic tubes containing 10 ml LB at a 1% dilution and were grown to an optical density of 0.6. Further steps were carried out in an anaerobic glove box or in ambient oxygen concentrations.

500 μl aliquots of culture were transferred to Eppendorf tubes to which different concentrations of arabinose were added. One set of Eppendorf tubes was incubated at 37 °C in a glove box under anaerobic conditions, and another set was incubated at 37 °C, aerobically for 1 h. Thereafter, 200 μl of the samples was added to each well of the microtiter plate.

The recombinant *B. longum*/pGBL8b strain was grown and treated essentially the same as the *E. coli* strain containing the reporter construct. The measurement of the reporter activity in anaerobic conditions is described below.

Light emission was measured using Hidex Chameleon multilabel reader (Turku, Finland) from 96-well microtiter plates that had been kept either in ambient atmosphere (*E. coli*/Sak-Lux plasmid), or in an anaerobic chamber (*E. coli*/pSak-Lux plasmid and *B. longum* NCC2755/pGBL8b). For *B. longum*, 10 μl of 1 mM D-luciferin (Aboatox Oy, Turku, Finland) in 0.1 M Na-citrate buffer, pH 5.0, was pipetted into the plate wells to start the light emission reaction. The bioluminescence emission from the cultures was measured after aerobic treatment to quantify the gene expression. A short incubation time (from 5 min on) under ambient atmosphere was adequate for the bioluminescence detection from anaerobic cultures, and this bioluminescence closely paralleled that of aerobically grown *E. coli*/pSak-Lux cells. *B. longum*/pGBL8b cells from anaerobically grown cultures were measured essentially in the same way as with *E. coli*. The measurements were performed directly from unbroken, living cells.

### 2.4. Dilution tests

pSak-Lux plasmid-containing *E. coli* XL1 cells were inoculated into anaerobic tubes and incubated O/N at 150 rpm at 37 °C. The next day, 1% of the O/N culture was inoculated to another anaerobic tube and incubated at 37 °C/150 rpm until it reached an $O.D._{600\ nm}$ of 0.6. Serial dilutions were prepared from the 0.6 O.D. culture in different anaerobic tubes in the glove box (anaerobic chamber). The dilutions were as follows: 1; 0.1; 0.01; 0.001; 0.0001; 0.00001). A stock solution of 1% arabinose was added to each Eppendorf tube (containing 500 μl of the

sample, 3 tubes each per sample) and the tubes were incubated at 37 °C in the glove box for 1 h. Next, the tubes were removed and a 200 µl sample from each Eppendorf tube was added to microtiter plate wells (3 replicates for each sample) and 10 µg/ml of tetracycline was added. The plates were read for 20 min at 5-min intervals.

The *B. longum*/pGBL8b cells were diluted and measured essentially the same as the *E. coli* reporter cells.

## 3. Results

*E. coli* XL-1 cells containing the plasmid pSak-Lux (map in Fig. S1), a derivative of plasmid pCGLS-11 (Frackman et al., 1990), which encodes a bacterial luciferase operon, was used as a model facultative anaerobic Gram (-) bacterial strain to study the reporter system in anaerobic conditions. As a Gram (+) model bacterium, we used *Bifidobacterium longum* NCC2705 that was transformed with the pGBL8b shuttle vector (Guglielmetti et al., 2008) containing an insect luciferase gene from a click beetle (*P. plagiophthalamus*) under the control of a strong phage T5 promoter.

The experimental data collected from the measurements of the insect luciferase reporter system confirmed that $O_2$ is strictly necessary for the luciferase/luciferin reaction as shown in logarithmic phase *B. longum*/pGBL8b cells. Nevertheless, oxygen was shown to rapidly diffuse into the culture medium and into the reporter cells after removing the plate from the anaerobic hood, even when all of the reagents were prepared and mixed under stringent anaerobic conditions. Furthermore, the addition of 0.2 volumes of D-luciferin solution prepared in an oxygenic environment to the bacterial broth culture supplied sufficient oxygen to detect significant light production, which showed saturation after approximately 40–50 min of incubation at room temperature (Fig. 1). Hereafter, the light emission measurements were performed 40 min after substrate addition.

The light emission as a function of cell density was studied by diluting the cells and measuring the bioluminescence of anaerobically grown *B. longum*/pGBL8b. Fig. 2 shows that even as few as approximately $2 \times 10^3$ log-phase-growing cells/ml can be detected via their light emitting activity. This is due to the use of a very strong phage T5 promoter (Reynolds et al., 1986) in front of the insect luciferase reporter gene. These results clearly indicate the convenience of using these powerful, but never-used, reporters in anaerobic organisms. The data show that the light emission is directly proportional to the cell count, also highlighting the wide dynamic measurement range in cell densities over several orders of magnitude. This sensitivity allows for the use of different types of light-emission measurement devices with various light gathering efficiencies, including luminometers, multilabel

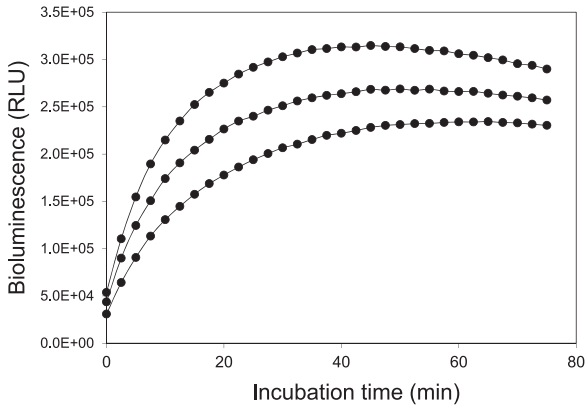


**Fig. 1.** Time curve for anaerobically grown *Bifidobacterium longum*/pGBL8b after removal from the anaerobic hood. The substrate for the luciferase reporter protein, D-luciferin, was added aerobically immediately after the anaerobic incubation of the cells, and the light-emission was continuously monitored in the plate reader.

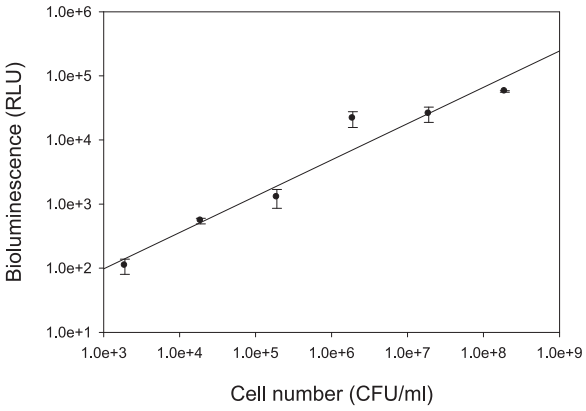


**Fig. 2.** Emission of light as a function of cell count in *B. longum*/pGBL8b. Vertical bars at each point represent the standard deviation of the data obtained from three independent measurements conducted in quadruplicate.

and scintillation counters, and even Polaroid film. The measurements were performed directly from unbroken, living cells, making the approach even more robust than the conventional ß-galactosidase-based reporter systems, which require cell disruption steps. The measurement of light emission from intact cells is possible due to the lowering of the pH to acidic values, as shown by Wood and DeLuca (1987), under which the substrate for insect luciferase, D-luciferin, is in its protonated form and can easily cross through the *E. coli* membranes. We have previously shown that this technique is also useful for other bacterial strains, such as *Streptococcus mutans* (Loimaranta et al., 1998) and *Staphylococcus aureus* (Tenhami et al., 2001).

Similar experiments were performed with the laboratory bacterium *E. coli* grown in anaerobic and aerobic conditions. The *E. coli* XL-1 strain with the bacterial luciferase operon in the plasmid pSak-Lux (*luxCDABE* from *P. luminescens)* allows the monitoring of gene expression without any addition of exogenous substrates, since the products of the operon produce the long chain aldehyde needed for light emission. The *lux* operon, containing the *luxCDABE*-genes, was inserted under the control of the arabinose promoter and the light-emission was monitored after arabinose induction in both aerobic and anaerobic conditions. As expected, the induction of protein expression is not affected by the absence of oxygen. Reporter proteins are expressed according to the concentration of the sugar used (in top right corner of Fig. 3). Five minutes of exposure to ambient oxygen, after the withdrawal of the plate from the anaerobic hood, is enough to get a saturated level of light emission for gene expression analysis in a microtiter plate-based measuring system. The decrease in light emission after five minutes at ambient air may be due to the flavin reductase coupled to NAD(P)H mediated enzymes upon oxygen starvation is not capable of producing enough $FMNH_2$ for the luciferase reaction (see Introduction for the luciferase reaction scheme). Compared to the bifidobacterial strain used in this study, *E. coli* is much faster in producing light, presumably due to the different luciferase reporter. In *B. longum*, the insect luciferase substrate D-luciferin must first pass the cytoplasmic membrane to reach the active centre of the reporter protein to achieve light emission. This delay as seen in Fig. 1 has also been noticed earlier in aerobically grown whole-cell bacterial reporter systems from several different types of microbial strains, from bacteria to yeasts (Lampinen et al., 1995; Leskinen et al., 2003).

A dilution set of several orders of magnitude was done to the reporter *E. coli* cells induced with 1% arabinose. The diluted cells were measured for RLU as a function of conventional plate counting. The light emitted is directly proportional to the amount of reporter cells (Fig. 4).

In addition, the induction of gene expression is not affected by whether the *E. coli* cells with the arabinose-inducible system are grown aerobically or anaerobically (Fig. 5 and Fig. 6). The induction closely

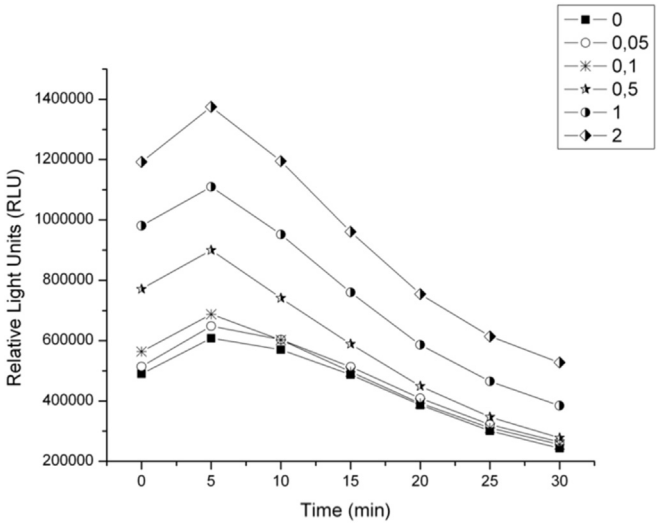


**Fig. 3.** Light emission by *E. coli* XL-1/pSak-lux from anaerobically grown cells induced with different concentrations of arabinose (% in the right corner box) as a function of time after aerobic incubation. The values are the means of two independent experiments conducted in triplicates. The variation between the replicates was less than 10%.

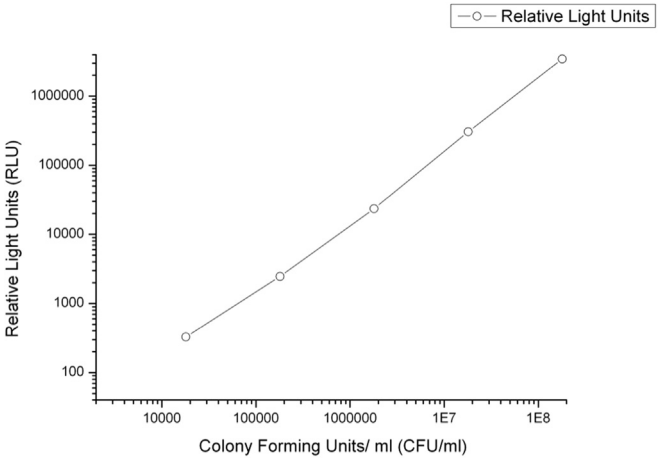


**Fig. 4.** Emission of light as a function of cell count in anaerobically grown *E. coli* XL-1/pSAK-Lux. The values are the means of two independent experiments conducted in triplicates. The variation between the replicates was less than 10%.

parallels the concentration of the inducer, arabinose, regardless of the incubation atmosphere. Here, the bacterial luciferase again shows a faster response to a change to anaerobic conditions compared to the insect luciferase.

## 4. Discussion

All organisms capable of emitting light as a by-product of their metabolism, bioluminescence, need oxygen for the reaction catalysed by the luciferase enzyme. Bioluminescent organisms exist on all of the continents of the globe, and include planktonic bacteria, algae, shrimps, fish and jelly fish in the sea, as well as terrestrial organisms, with the *Photinus pyralis* beetle being the most well-known example. One bacterial species, *P. luminescens*, is the only example of a terrestrial bacteria capable of bioluminescence. Sometimes, fluorescence goes hand-in-hand with bioluminescence in nature. This relationship was originally demonstrated by Osamu Shimomura in his studies with a luminous hydromedusan, *Aequorea victoria*, from which he was able to extract

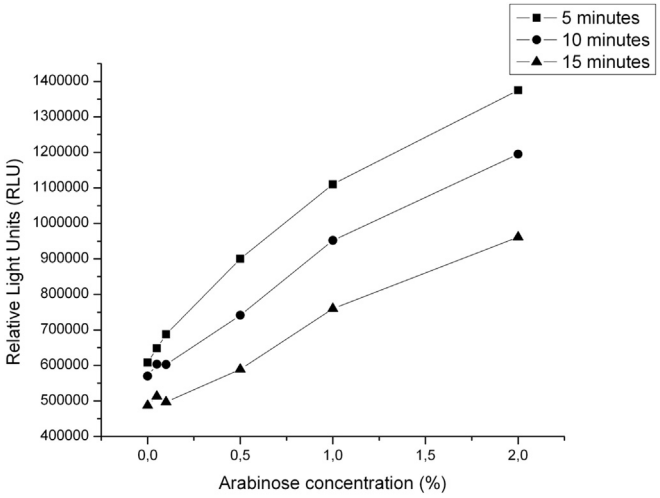


**Fig. 5.** Emission of light as a function of the concentration of the inducer, arabinose, after transferring the anaerobically grown cells to the ambient oxygen concentration. The values are the means of two independent experiments conducted in triplicates. The variation between the replicates was less than 10%.

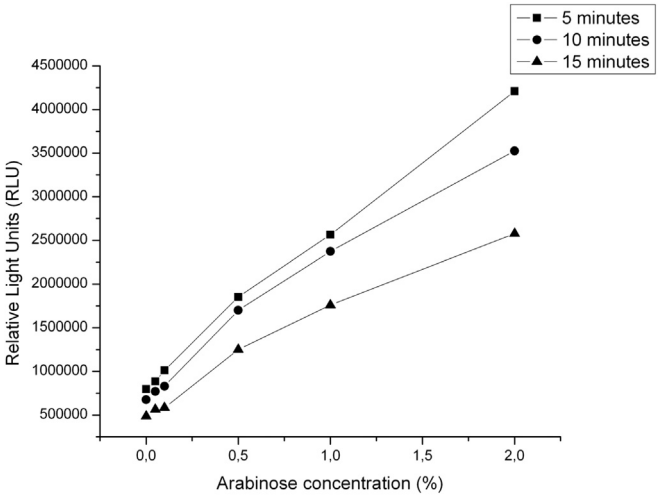


**Fig. 6.** Emission of light as a function of the concentration of the inducer, arabinose, after growing the cells aerobically. The different time points represent the relatively light units (RLU) after placing the microtiter plate into the plate counter. The values are the means of two independent experiments conducted in triplicates. The variation between the replicates was less than 10%.

and purify two activities, a fluorescent protein that was excited by a bioluminescent enzyme (Shimomura et al., 1962). More and more organisms capable of producing fluorescence upon excitation have been found, and their fluorescence emission and mode of action have been characterized. Furthermore, the proteins responsible for fluorescence have been mutated to change, for instance, the emission wavelength, making it possible to normalize experiments by measuring two or more different spectra at the same time. Fluorescent proteins require oxygen for proper folding of the chromophore inside the can-like structure of the protein to produce fluorescence light; on the contrary, bioluminescent proteins (luciferases) are enzymes (Tsien, 1998). The mCherry fluorescent protein was used as a bioreporter in a recent study, and it was shown to also work in anaerobic conditions (Muhr et al., 2015). Sixty minutes of incubation in ambient oxygen (air) was sufficient to ensure maturation for microscopic analysis. However, in this report no optimizations or quantitative analysis were shown. Newer fluorescent proteins that don't require molecular oxygen have recently been found

for studies in anaerobic or microaerobic conditions (Landete et al., 2015). It was found that the plasmid evoglow-Pp1 (Evocatal GmbH, Germany) allowed fluorescence generation in anaerobic conditions. However, fluorescence was affected by external pH in lactobacilli. Furthermore, a pitfall in the use of fluorescent proteins is that all cells, from prokaryotic to eukaryotic organisms, emit intrinsic fluorescence. This property causes a background fluorescence level that should be taken into account by subtracting the fluorescence of cells carrying the empty plasmid. This issue lowers the detection limit of the system to nanomolar levels and causes extra work (Hakkila et al., 2002). Recently, a flavin mononucleotide (FMN)-based fluorescent protein (FbFP) was described as reporter for anaerobic promoter screening in *Clostridium cellulolyticum* (Teng et al., 2015). The authors showed that is possible to measure promoter activity in situ from anaerobically growing clostridial strains. However, they did not mention the sensitivity of the system, and, according to the graphs, the dynamic measurement range was only a few hundred fluorescence units, while luciferase-based reporters have a range of hundreds of thousands of relative light emission units (see Fig. 2 and Fig. 4).

Cronin et al. (2008) constructed a bacterial luciferase cassette to study *Bifidobacterium breve* persistence in living mice. They took into account the possible absence of oxygen needed for the light emission reaction in the mouse gut and found that sufficient aerobic conditions existed for the luciferase reaction to occur. They did not further study the role of oxygen in this living animal setup.

Bacterial luciferase reporter genes have also been used in several other applications. An *E. coli mer-luc* bioreporter was used in anaerobic conditions to monitor the intracellular accumulation of trace concentrations of Hg(II) ions (Golding et al., 2008). All of the incubations of the cells were performed in anaerobic conditions, and the actual reporter gene activity analysis was done by opening the cap of the incubation tube for three minutes to aerate the sample, followed by quick measurement in a liquid scintillation counter. They concluded that this approach would be unlikely to have an effect on the bioreporter response, because it was assumed to take 20 min for the bacteria to sufficiently express the bioluminescence reporter proteins to detectable levels. However, they did not further study or optimize the conditions for the bioluminescence reaction after anaerobic growth conditions.

Bioluminescent *Mycobacterium tuberculosis* was made by transferring the *luxAB* reporter genes into strain ATCC27294 (Cho et al., 2007). Light emission was obtained from cells cultured in low oxygen conditions by adding the substrate n-decanal, and the emission was correlated to the antibiotic dosage used to cure the pathogenic bacterium. As in the previous study, the oxygen-dependent reporter was successfully utilized in anaerobic conditions; however, the conditions were not thoroughly optimized for the reporter's performance. The *luxAB* luciferase reporter genes from the bacterium *Aliivibrio fischeri* were used to monitor bioluminescence expression in the anaerobe *Clostridium perfringens* (Phillips-Jones, 1993). The author further expanded the study to monitor rapid changes in α-toxin gene expression in the same host to correlate it to reporter gene expression (Phillips-Jones, 2000). She found that the reporter gene expression closely paralleled the α-toxin expression and that measurement of *lux*-gene expression provided a rapid and real-time non-invasive measurement of gene expression. However, she had to add the bacterial luciferase substrate n-decanal similar to in the study of Cho et al. described above.

There is no robust and universal reporter gene system for studies of gene expression and regulation in anaerobic organisms. Nevertheless, anaerobic organisms represent the vast majority of all living creatures on Earth. Furthermore, these organisms allow man to manufacture high value chemicals, food and drink of various sorts by fermentation and to help purify water and municipal wastes. Here, we have shown that well-known light-emitting proteins, luciferases and their encoding genes, can be efficiently utilized as genetic reporters in Gram (+) and Gram (-) bacteria via short exposure to ambient oxygen and without extra cell disruption steps. Combined with sensitive measurement technologies with sophisticated electronics, this approach forms a bioreporter system that can be miniaturized to be suitable for field or in situ usage. The dynamic measurement range is several orders of magnitude due to the lack of background noise that disturbs other reporter systems utilized thus far.

## 5. Conclusions

Biosensors often utilize whole-cell, engineered cell systems in combination with sensitive devices to measure light emission. From the examples shown above, quite a few studies have used reporters that need oxygen for their activity measurements to successfully quantify gene expression in anaerobically grown bacterial cells. Common to all of these studies is that the phenomenon itself was not studied and characterized in detail. In our study, we compared two luciferase reporter systems in two different bacterial strains. Taken together, the examples using two aerobic reporter systems in two anaerobically grown microorganisms showed that the real-time monitoring of gene expression in anaerobic microorganisms is robust and simple, even though it was previously considered impossible. The reported methodology is universal for bacteria and probably also applies to yeasts and fungi. We have shown that the above approach can be used for the measurement of gene expression both in Gram (+) and Gram (-) bacterial strains using insect and bacterial luciferase reporters. Future gene expression studies of anaerobic microorganisms will prove the strength of our approach.

## Acknowledgements

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bios.2018.09.066.

## References

Cecic, I., Chan, D.A., Sutphin, P.D., Ray, P., Gambhir, S.S., Giaccia, A.J., Graves, E.E., 2007. Mol. Imaging 6 (4), 219–228.
Cho, S.H., Warit, S., Wan, B., Hwang, C.H., Pauli, G.F., Franzblau, S.G., 2007. Antimicrob. Agents Chemother. 51 (4), 1380–1385.
Cronin, M., Sleator, R.D., Hill, C., Fitzgerald, G.F., van Sinderen, D., 2008. BMC Microbiol. 8, 161.
Daunert, S., Barrett, G., Feliciano, J.S., Shetty, R.S., Shrestha, S., Smith-Spencer, W., 2000. Chem. Rev. 100 (7), 2705–2738.
Drepper, T., Eggert, T., Circolone, F., Heck, A., Krauss, U., Guterl, J.K., Wendorff, M., Losi, A., Gartner, W., Jaeger, K.E., 2007. Nat. Biotechnol. 25 (4), 443–445.
Edwards, A.N., Pascual, R.A., Childress, K.O., Nawrocki, K.L., Woods, E.C., McBride, S.M., 2015. Anaerobe 32, 98–104.
Elad, T., Lee, J.H., Belkin, S., Gu, M.B., 2008. Microb. Biotechnol. 1 (2), 137–148.
Feustel, L., Nakotte, S., Durre, P., 2004. Appl. Environ. Microbiol. 70 (2), 798–803.
Frackman, S., Anhalt, M., Nealson, K.H., 1990. J. Bacteriol. 172 (10), 5767–5773.
Golding, G.R., Sparling, R., Kelly, C.A., 2008. Appl. Environ. Microbiol. 74 (3), 667–675.
Guglielmetti, S., Ciranna, A., Mora, D., Parini, C., Karp, M., 2008. Int. J. Food Microbiol. 124 (3), 285–290.
Guglielmetti, S., Karp, M., Mora, D., Tamagnini, I., Parini, C., 2007. Appl. Microbiol. Biotechnol. 74 (5), 1053–1061.
Hakkila, K., Maksimow, M., Karp, M., Virta, M., 2002. Anal. Biochem. 301 (2), 235–242.
Hansen, M.C., Palmer Jr., R.J., Udsen, C., White, D.C., Molin, S., 2001. Microbiology 147 (Pt 5), 1383–1391.
Lampinen, J., Virta, M., Karp, M., 1995. Appl. Environ. Microbiol. 61 (8), 2981–2989.
Landete, J.M., Langa, S., Revilla, C., Margolles, A., Medina, M., Arques, J.L., 2015. Appl. Microbiol. Biotechnol. 99 (16), 6865–6877.
Leskinen, P., Virta, M., Karp, M., 2003. Yeast 20 (13), 1109–1113.
Loimaranta, V., Tenovuo, J., Koivisto, L., Karp, M., 1998. Antimicrob. Agents Chemother. 42 (8), 1906–1910.
Michelini, E., Cevenini, L., Mezzanotte, L., Ablamsky, D., Southworth, T., Branchini, B., Roda, A., 2008. Anal. Chem. 80 (1), 260–267.
Muhr, E., Leicht, O., Gonzalez Sierra, S., Thanbichler, M., Heider, J., 2015. Front. Microbiol. 6, 1561.

Phillips-Jones, M.K., 1993. FEMS Microbiol. Lett. 106 (3), 265–270.
Phillips-Jones, M.K., 2000. FEMS Microbiol. Lett. 188 (1), 29–33.
Reynolds, A.E., Mahadevan, S., LeGrice, S.F., Wright, A., 1986. J. Mol. Biol. 191 (1), 85–95.
Rizzotti, M., 2009. In: UNESCO (Ed.).
Selifonova, O., Burlage, R., Barkay, T., 1993. Appl. Environ. Microbiol. 59 (9), 3083–3090.
Shimomura, O., Johnson, F.H., Saiga, Y., 1962. J. Cell. Comp. Physiol. 59, 223–239.
Teng, L., Wang, K., Xu, J., Xu, C., 2015. J. Microbiol. Methods 119, 37–43.
Tenhami, M., Hakkila, K., Karp, M., 2001. Antimicrob. Agents Chemother. 45 (12), 3456–3461.
Tsien, R.Y., 1998. Annu. Rev. Biochem. 67, 509–544.
Whitman, W.B., Coleman, D.C., Wiebe, W.J., 1998. Proc. Natl. Acad. Sci. USA 95 (12), 6578–6583.
Wood, K.V., DeLuca, M., 1987. Anal. Biochem. 161 (2), 501–507.