Journal of Microbiological Methods 118 (2015) 78–80

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Note

Method with high-throughput screening potential for antioxidative substances using *Escherichia coli* biosensor *katG′::lux*

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ARTICLE INFO

Article history:
Received 16 June 2015
Received in revised form 19 August 2015
Accepted 24 August 2015
Available online 29 August 2015

Keywords:
Antioxidative activity
Bacterial biosensor
Bioscreening
Microplate technique

ABSTRACT

A new method is described for the rapid real-time screening of antioxidative properties using a recombinant *Escherichia coli* DPD2511 biosensor. This microplate technique, without time-consuming pre-incubations and handling, has potential for a high-throughput search of bioactive compounds. Special emphasis was given to obtaining highly reliable and repeatable results.

When using intact living biosensors, both bioactivity and bioavailability of a chosen sample material can be detected simultaneously in a simple, cost-effective and rapid manner, which makes the assays more suitable for high-throughput screening (HTS) (Galluzzi and Karp, 2006). These effects can be monitored continuously and without long incubation or handling times. The *Escherichia coli* strain DPD2511 (Belkin et al., 1996) has been constructed by fusing the *katG* (catalase) genes of the strain to the luminescence (*lux*) genes from the *Vibrio fischeri* bacteria. The bioavailable oxidant is able to enter through the *E. coli* cell membrane and bind to the regulatory protein OxyR which promotes the transcription and translation of the reporter genes. With the *lux* gene fusion this produces an increase in luminescent light emission, which can be measured and quantified in a continuous manner (Michelini et al., 2005). Thus, the normal defense mechanism signaling against oxidizing agents produces an easily monitored response. When antioxidative activity is measured, the bacteria are exposed to an oxidant such as hydrogen peroxide (H_2O_2), and the experiment is set to measure whether samples prevent the stress reaction. In other words the method measures the inhibition capacity of the antioxidant against H_2O_2. The strain was used to screen antioxidative properties of medicinal plants used in the Philippines and validated against the DPPH assay by Bartolome et al. (2006). We describe a microplate technique of the screening method with an improved HTS potential.

The bacteria were preserved in 15% glycerol at −80 °C. Working stock was prepared by inoculating Luria Agar growth-plates supplemented with 100 μg/ml of ampicillin and 10 vol.% of 1 M potassium phosphate buffer. The plates were cultivated overnight at 30 °C before storing in 4 °C. Cultivations were discarded after a week from inoculation as they lose their sensitivity during prolonged storing (Kim and Gu, 2003). A single colony of the strain was inoculated into 5 ml of liquid Luria Broth medium supplemented with 100 μg/ml of ampicillin and 10 vol.% of 1 M potassium phosphate buffer and incubated for approximately 16 h in a shaker at 30 °C and 300 rpm, after which the luminescence was measured with a Chameleon Multilabel (Hidex Oy, Finland) microplate reader. The cell culture producing the highest signal was chosen for the measurement.

For the assay with H_2O_2, 100 μl of the chosen cell culture was added to each well of an opaque white microplate (Thermo Electron Corporation, Finland) containing 50 μl of fourfold dilutions of the H_2O_2 and 50 μl of sterile water. Sterile water was also used as a negative control. Luminescence was measured in counts per seconds (CPS) 20 times every 5 min and between the screenings the plate was shaken and kept at 30 °C. CPS values vary depending on the date and chosen culture. Thus, the results are expressed in induction factors (FI) calculated by dividing the CPS values of the samples by the value of the negative control. For the ascorbic acid (AA) measurements, a constant concentration of 4 mM of H_2O_2 was used in each well and 50 μl of sterile water was replaced with AA concentrations in the 1 M potassium phosphate buffer. Sterile water with the phosphate buffer was used as the negative control.

Tryptone, yeast extract and agar were obtained from Lab M Limited, UK. Sodium salt of ampicillin was obtained from Sigma-Aldrich, USA.

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http://dx.doi.org/10.1016/j.mimet.2015.08.018
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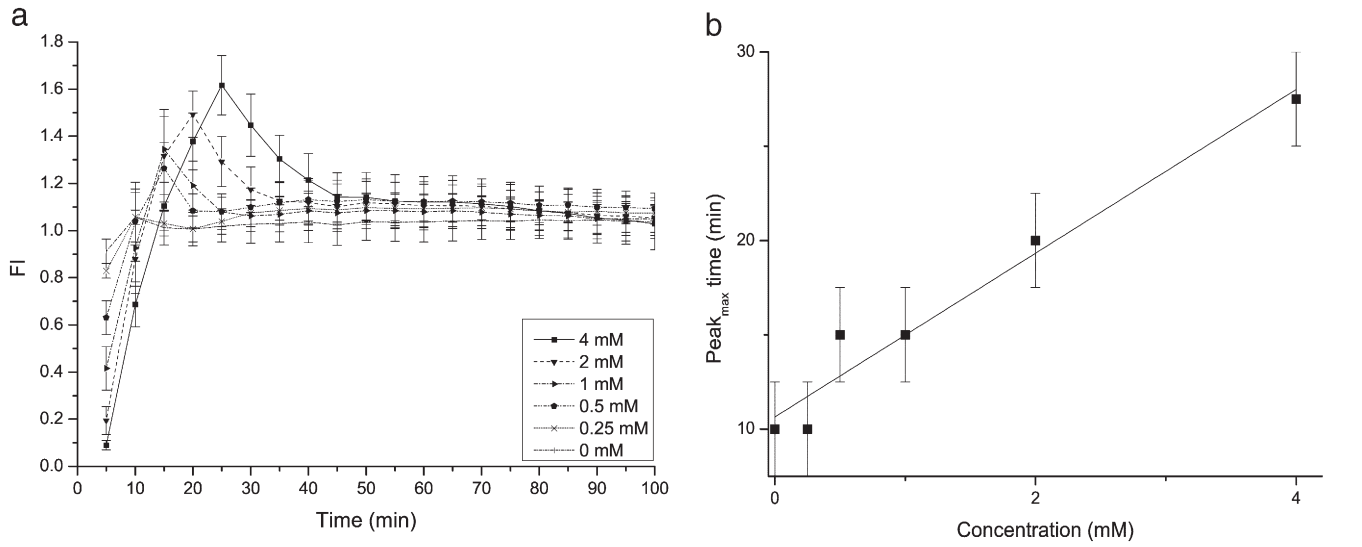

Fig. 1. (a) Luminescence produced by the H_2O_2 concentrations (mM) in FI represented with a 5 measurement average with CV% between measurements as the error bars. (b) The lag time before the peak maximum increases as the concentration increases. As the reading device measures the luminescence emitted every 5 min, the error bars are a constant ±2.5 min for every $peak_{max}$ time value.

Sodium chloride, KH_2PO_4 and H_2O_2 were from Merck KGaA, Germany and L-ascorbic acid and K_2HPO_4 were from VWR International, USA.

The results of the H_2O_2 assay are shown in Fig. 1a. The FI values were directly proportional to the sample concentration used but also the lag time before the FI maximum increased with the concentration (Fig. 1b). For this reason the experiment was continued for 100 min even though the rise in signal could be detected in less than 20 min. The temperature stabilized at 30 °C after the first 10–15 min and therefore the error before this time can give higher values. The coefficient of variation (CV%) of the four parallel concentrations on the plate was constantly under 10%. Repeatability of the method is also high as the CV% of the results between 5 alternate measurements gave no higher values than 10%. Because the concentration of 4 mM of H_2O_2 gave the highest factor of induction values, this concentration was selected for the antioxidative activity measurements.

The results for the first 50 min of the experiment with AA are shown in Fig. 2a. All of the AA concentrations were able to inhibit some of the light induction produced by the H_2O_2 concentration of 4 mM for 50 min. After this time the signals began to rise exponentially, which is probably due to the toxic effects of H_2O_2 overcoming the inhibition effect of the AA concentrations. In Fig. 2b the %inhibition (Eq. (1)) (Bartolome et al., 2006) of the AA concentrations is shown at the time point 25 min. The CV% of the sample quadruplicates in the microplate was not higher than 11% from 10 to 45 min. The highest value of error was produced by three of the highest concentrations at the time point 20 or 25 min. Otherwise it was well over 10% between the same time limits. At 50 min as the signal starts to rise, the error also rises.

$$\%\text{inhibition} = \frac{L_{solvent} - L_{ascorbic\ acid}}{L_{solvent}} \times 100 \quad (1)$$

The method was also used to screen antioxidative activities from various extracts (fungal mycelium extracts, Scots pine and Norway spruce tissue extracts and pure stilbene compounds) from Finnish forests and the example figure is presented in the supplementary figure. In conclusion, the results obtained in this study show that the developed method using recombinant *E. coli* harboring *katG′::lux* fusion is suitable

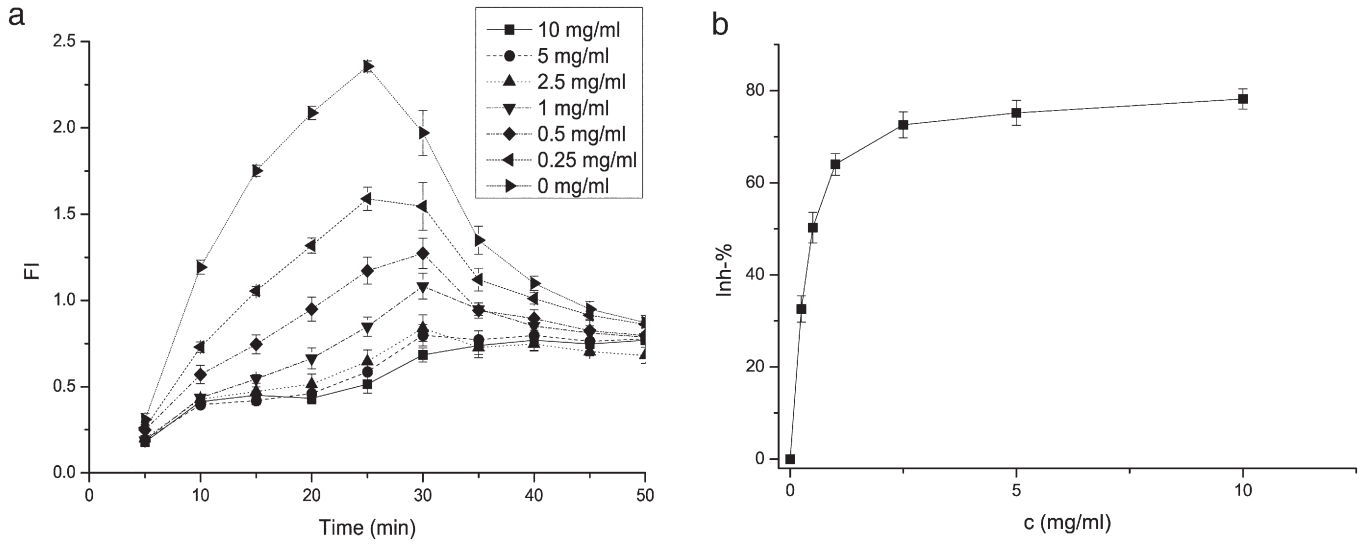

Fig. 2. (a) The antioxidative activity of the AA concentrations measured with the *E. coli* sensor. The error bars are the CV% of the sample quadruplicates in the microplate. (b) The %inhibition of AA shown at 25 min. The error bars are calculated from the standard deviation of the sample quadruplicates in the microplate.

to screen for the antioxidative properties of sample material in a simple, rapid and reliable manner, and it embodies real HTS potential.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

Dr. LaRossa has kindly provided the *Escherichia coli* DPD2511 strain. M. Karp acknowledges the sabbatical year funding from the Finnish Cultural Foundation (Suomen Kulttuurirahasto). Authors also acknowledge P. Fairchild for the language check of this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.mimet.2015.08.018.

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