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Digital camera-based lipase biosensor for the determination of paraoxon

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ABSTRACT

This work is focused on construction of a biosensor containing unique bacterial homogenate with high lipase activity and colorimetric type of assay where camera of a smartphone was chosen as a detector. The biosensor was constructed as a tool of lipase inhibitors and paraoxon served as a representative analyte inhibiting the lipase.

Psychrophilic strains of bacteria isolated from in Antarctica were tested and the best isolate P4368 having huge lipase activity was chosen. Tween assay was performed as a standard method for lipase activity determination and indoxylacetate served as a substrate of lipase measurable by smartphone camera and R, G and B color channels digital analysis. Bacteria were homogenized and immobilized on polyvinylidene difluoride membrane and indoxylacetate was immobilized in the proximity of the homogenate. Paraoxon ethyl was analyzed by the standard method and by the camera-based biosensor.

The biosensor-based assay was found to determine paraoxon with limit of detection 3.72×10^{-8} mol/l and IC50 value for paraoxon was 4.00×10^{-6} mol/l. A volume 5 µl of sample was sufficient for the assay and the sample was applied directly without any processing.

In a conclusion, a simple colorimetric biosensor for the determination of venomous compounds like organophosphorus pesticides and nerve agents was constructed and promising analytical parameters were received during its characterization.

1. Introduction

Lipases are enzymes which have been applied in biotechnology industry since 1980s [1]. In biochemical databases, the enzymes are enrolled as triacylglycerol acylhydrolases (EC 3.1.1.3) responsible for hydrolysis of triglycerides to partial glycerides and fatty acids but the other esters can be hydrolyzed as well [2,3]. Lipases belong to a wider group known as serine hydrolases. The name indicates the fact that their active site include serine and in a wider look histidine and aspartate or glutamate are also amino acids important for the enzyme activity [2]. In microorganisms, lipases can be located as extracellular as well as intracellular one. *Rhizopus homothallicus, Aspergillus carneus* and *Williopsis californica* have extracellular activity of lipases while yeast *Saccharomyces cerevisiae* keep major activity inside the cell [4].

A wide number of lipases technological applications are known. Namely the application where esters bond is hydrolyzed, trans- or interesterification takes places are typical chemical reaction catalyzed by lipases in the current biotechnology. Production of vegetable oils [5], production of geranyl acetate, isoamyl butyrate, benzyl propionate for cosmetic industry [6], fabrication of biofuels by processing of methyl esters of fatty acids like methyl palmitate and methyl oleate [7] can be exampled. In the field of analytical chemistry, lipases were recognized as a material for biosensors construction where the enzymes played a role of biorecognition part selective to analyte. The detection of organophosphate pesticides by biosensors with immobilized lipase described in some papers [8–12].

This paper is devoted to construction of a biosensor with unique bacteria having high activity of lipase. Unique strains of bacteria coming from Antarctica were chosen as a source of the enzymes and the biosensor was constructed using just this specific material. Digital camera of a smartphone is considered as a measuring device allowing applicability. It is intended to found a reliable analytical tool that will become easily reach in praxis working on another principle and material than are the current technologies. This work is represented on paraoxon as an analyte but the analyte is chosen as an example compound. Practical impact of this approach is expected.

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2. Materials and methods

2.1. Microorganisms

Sampling sites were situated in a deglaciated northern part of the James Ross Island, Antarctica. Sampling was carried out by dispersing 1 g of soil sample in 5 ml of sterile saline solution, and 200 µl of this suspension was spread by L-loop on the surface of an R2A (Oxoid) agar plate and cultivated at 15 °C for up to 5 days. Afterwards, individual morphologically different colonies were continuously picked up, purified by repeated streaking on R2A medium at 15 °C, and the obtained pure cultures were maintained at – 70 °C until analysed. The lipase activity was tested on agar medium by Páčová and Kocur [13] and from almost three hundreds strains with positive hydrolysis of Tween 80, the strain P4368 was chosen as the best producer of huge amount of lipase activity. Strain P4368 was isolated from surface of small pieces of stones, locality Santa Martha Slopes. Based on 16S rDNA sequencing, the P4368 was identified as *Psychrobacter* sp.

2.2. Cells processing and immobilization procedure for the biosensor construction

The cultured cells were collected and resuspended in phosphate buffered saline to achieve approximate concentration 10^9 cells/ml. The cells were sonicated using ultrasound homogenizer Ultrasonic UP50H (Hielsher Ultrasonics, Teltow, Germany). For the homogenization purposes, ultrasound probe was immersed into 1 ml of bacterial suspension in a tube placed in an ice bath. The suspension was sonicated on for 30 s for three times. One-minute interval of rest for cooling the suspension down was placed between the sonication periods. Total protein in the bacterial homogenate was determined by Bradford method using total protein kit TP0100 (Sigma-Aldrich, Saint Louis, Missouri, USA) and absorbance assay 595 nm as recommended in the attached instruction manual.

Polyvinylidene difluoride membrane with 0.45 µm pore size (Sigma-Aldrich) served as a matrix for cell homogenate immobilization. Chitosan was chosen for stabilizing the biorecognition element on the matrix surface. Prior to the immobilization, chitosan (Litolab; Chudobin; Czech Republic) in an amount 18 mg was solved in 0.2 mol/l acetic acid. In a total 10 µl of bacterial homogenate and 10 µl of chitosan solution was poured and mixed on surface of polyvinylidene difluoride membrane by suck in and let out from micropipette. A dot comprised of 20 µl indoxylacetate (Litolab) in ethanol (concentration was an object of optimization) was made in the proximity of the dot with bacterial homogenate but the dots were kept separated. Finally, the membranes were dried and stored until use under standard ambient laboratory temperature and pressure (25 °C, 100 kPa) in the dark.

2.3. Standard determination of lipase activity using tween

Lipase activity determination was made in compliance with the cited protocols [14,15] and slightly modified. Buffer containing tween 20 (in chemical terminology polysorbate 20; purchased from Sigma-Aldrich) with calcium chloride 80 mmol/l and 20 mmol/l Tris-HCL buffer pH 7.0 (further lipase activity buffer) was poured with sample bacterial sample in a cuvette with optic path length 1 cm. In a total 990 µl of the lipase activity buffer and 10 µl of bacterial homogenate were poured. When inhibition by paraoxon ethyl investigated, a solution of paraoxon ethyl (Sigma-Aldrich) in dimethyl sulfoxide in an amount 100 µl was poured with 890 µl of the lipase activity buffer and 10 µl of bacterial homogenate. After that, cloudiness of the formed calcium laurate was measured at 450 nm after an incubation lasting 20 min. Pure lipase activity buffer without added bacterial homogenate was used as a blank.

2.4. Indoxylacetate assay in cuvette

Indoxylacetate was used as a chromogenic and fluorogenic substrate for lipases. The assay was made in a cuvette with optic path length 1 cm. Indoxylacetate was dissolved in ethanol and in a total 25 µl of indoxylacetate solution (concentration was an object of optimization) was injected into cuvette and then phosphate buffered saline pH 7.4 was added in an amount 965 µl. The reaction was started by 10 µl of bacterial homogenate. Blue coloration was measured at 670 nm after an incubation lasting 20 min. Solution containing the aforementioned solutions with indoxylacetate and bacterial homogenate replaced by phosphate buffered saline was used as a blank.

2.5. Camera based assay

The camera-based assay was done under standard ambient laboratory temperature and pressure (25 °C, 100 kPa). The assay was started by injection of a phosphate buffered saline over the sites were the dot with bacterial homogenate and the dot with indoxylacetate were originally placed. In a total 50 µl of buffer or a solution containing 45 µl of buffer and 5 µl of paraoxon ethyl in dimethyl sulfoxide was applied and let to incubate for 20 min and covered with an extension tube made from black acrylonitrile butadiene styrene using 3D printer Prusa i3 (Prusa Research; Prague, Czech Republic). After the incubation period, the smartphone Samsung Galaxy S5 (Seoul, South Korea) was placed on the extension tube and pictures of dots were taken under illumination of LED flash integrated in the smartphone. The extension tube was high 15 cm which determined constant geometry between the smartphone and the dot, and the black color of the tube in combination with white surface of the polyvinylidene difluoride membrane allowed constant setting of white balance. Principle of the biosensor function can be learned from Fig. 1.

2.6. Data processing

All measurements were made in pentaplicate and the both average value and standard deviation were calculated for each of group of same samples and/or experimental conditions. Software Origin 9 (OriginLab Corporation, Northampton, MA, USA) was chosen for statistical processing of the achieved data. Signal to noise equal to three (S/N = 3) was applied as a rule for limit of detection calculation. Comparison of different groups of samples for their difference was made by analysis of variance (ANOVA) on the both $P = 0.05$ and $P = 0.01$ probability levels. Michaelis constant K_M was calculated using Origin 9 by non-linear curve fitting with Michaelis Menten equation.

Images processing was made as follow. Captured photographs were opened in an editor application of Zoner Photo Studio 17 PRO (Brno; Czech Republic) software and color shift function was chosen for color depths acquiring. Using the color shift function, the color depths for all three channels (R, G and B) were read in a region on the photograph responding to half of a semi-diameter of the dot on paper matrix. The images were taken in 8 bit jpg format and color depths can theoretically reach value 0–255 which represents theoretical possible 256 variations for each channel. Principle of the photography assay can be learned from cited papers [16,17].

3. Results and discussion

The bacterial homogenate was tested for the standard enzyme kinetics and total protein content prior to construct the biosensor. Tween 20 was chosen as a standard substrate for bacterial lipases considering literature search [18]. Michaelis constant for tween 20 was equal to 217 ± 26 µmol/l. Total protein content was determined to be 0.405 ± 28 mg/ml (milligrams of protein per milliliter of homogenate). The Michaelis-Menten kinetics for tween 20 is depicted as Fig. 2. Concentration of tween 20 equal to 2 mmol/l was selected as