Journal of Biotechnology 267 (2018) 45–49

Contents lists available at ScienceDirect

Journal of Biotechnology

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

Short communication

Smelling *Pseudomonas aeruginosa* infections using a whole-cell biosensor – An alternative for the gold-standard culturing assay

Igor Kviatkovski[a], Sagit Shushan[b,c], Yahav Oron[c,d], Idan Frumin[b], Daniel Amir[b], Lavi Secundo[b], Eitan Livne[b], Aharon Weissbrod[b], Noam Sobel[b], Yael Helman[a,*]

[a] Department of Plant Pathology and Microbiology, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100, Israel
[b] Department of Neurobiology, Weizmann Institute of Science, Rehovot 76100, Israel
[c] Department of Otolaryngology-Head and Neck Surgery, Edith Wolfson Hospital, Holon, Israel
[d] Tel-Aviv Sourasky Medical Center, Tel-Aviv University, Israel

ARTICLE INFO

Keywords:
Pseudomonas aeruginosa infection
Otitis externa
2-Aminoacetophenone
Whole-cell biosensor

ABSTRACT

Improved easy-to-use diagnostic tools for infections are in strong demand worldwide. Yet, despite dramatic advances in diagnostic technologies, the gold-standard remains culturing. Here we offer an alternative tool demonstrating that a bacterial biosensor can efficiently detect *Pseudomonas aeruginosa* infections in patients suffering from otitis externa. Detection was based on specific binding between the biosensor and 2-aminoacetophenone (2-AA), a volatile produced by *P. aeruginosa* in high amounts. We collected pus samples from ears of 26 subjects exhibiting symptoms of otitis externa. Detection of *P. aeruginosa* using the biosensor was compared to detection using gold-standard culturing assay and to gas-chromatograph–mass-spectrometry (GC–MS) analyses of 2-AA. The biosensor strain test matched the culture assay in 24 samples (92%) and the GC–MS analyses in 25 samples (96%). With this result in hand, we designed a device containing a whole-cell luminescent biosensor combined with a photo-multiplier tube. This device allowed detection of 2-AA at levels as low as 2 nmol, on par with detection level of GC–MS. The results of the described study demonstrate that the volatile 2-AA serves as an effective biomarker for *P. aeruginosa* in ear infections, and that activation of the biosensor strain by 2-AA provides a unique opportunity to design an easy-to-use device that can specifically detect *P. aeruginosa* infections.

1. Introduction

Pseudomonas aeruginosa is an opportunistic human pathogen capable of infecting various tissue types (Bjarnsholt et al., 2009; Gómez and Prince, 2007; Streeter and Katouli, 2016; Wang et al., 2005). It is notorious for its persistent antibiotic-resistant mucoid biofilms, and it is well established that early aggressive antibiotic treatment of its infections is imperative for successful treatment (Bjarnsholt et al., 2009). Currently, routine detection of *P. aeruginosa* infections is carried out by culture inoculation. This method, which can detect *P. aeruginosa* in one to three days, requires analyses of samples in specialized laboratories and is prone to false results (Brown and Lowbury, 1965; Xu et al., 2004). The necessary growth step of bacteria in the culturing method can generate false-positive results due to contamination of plates and medical devices, as well as false-negative results due to overgrowth by other bacteria in a mixed-species infection (Verenkar et al., 1993; Burman and Reves, 2000; Xu et al., 2004). Therefore, new tools for faster and more reliable culture-independent detection of *P. aeruginosa* are in demand, and methods based on polymerase chain reaction (PCR), serological analysis or detection of volatile compounds emitted by *P. aeruginosa* have been examined (Goeminne et al., 2012; Héry-Arnaud et al., 2016; Savelev et al., 2011; Spilker et al., 2004; Tramper-Stranders et al., 2006; Xu et al., 2004). Though these methods may be more accurate than the gold-standard culturing assay, they all require specialized equipment, laboratories and personnel, and therefore cannot be easily implemented in clinics.

Several studies reported that the production of 2-aminoacetohenone (2-AA), a low-weight volatile compound produced by *P. aeruginosa* in high amounts, could be used as a biomarker for the presence of *P. aeruginosa* in infected tissues (Preti et al., 2009; Scott-Thomas et al., 2010). Detection of 2-AA and other *P. aeruginosa* volatiles has thus far been carried out by elaborate gas-chromatograph mass-spectrometry

Abbreviations: 2-AA, 2-aminoacetophenone; PMT, photo multiplier tube; GC–MS, gas chromatography mass–spectrometry

* Corresponding author at: Department of Plant Pathology and Microbiology, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76100, Israel.
E-mail address: yael.helman@mail.huji.ac.il (Y. Helman).

https://doi.org/10.1016/j.jbiotec.2017.12.023
Received 15 August 2017; Received in revised form 7 December 2017; Accepted 27 December 2017
Available online 29 December 2017
0168-1656/

(GC–MS) or selected-ion flow-tube mass-spectrometry (SIFT-MS) (Carroll et al., 2005; Goeminne et al., 2012). These methods are costly and require technical experience, both often lacking in local medical centers. Here we suggest an alternative method for detection of 2-AA. We have previously reported that 2-AA specifically activated the LuxR quorum sensing (QS) receptor of the marine bacterium *Aliivibrio fischeri* (Kviatkovski et al., 2015). We found that 2-AA activated the LuxR receptor of both wild type *A. fischeri* MJ-1 and the genetically modified *Escherichia coli* MJ109/pSB401 strain. The latter, which was originally constructed by Winson et al. (1998) to respond to acyl-homoserine lactone (the non-volatile cognate signal of LuxR), harbors the pSB401 plasmid encoding the LuxR receptor in conjugation to the luciferase encoding operon. We showed that the *E. coli* MJ109/pSB401 strain (thereafter named the biosensor strain), was activated upon exposure to *P. aeruginosa*'s volatile bouquet and specifically to 2-AA (Kviatkovski et al., 2015). According to our previously reported experiments and *in silico* docking analysis, the specific activation of the LuxR receptor by 2-AA was dependent on the positions of the amine and ketone in the 2-AA ring and was based on the interaction of 2-AA with seven amino acids within the binding pocket of the receptor (Kviatkovski et al., 2015).

Activation of LuxR by 2-AA was deemed highly specific because (i) LuxR did not respond to 2-AA homologs, (ii) 2-AA did not activate other LuxR-like receptors, and (iii) volatiles of 2-AA deficient *P. aeruginosa* mutants did not activate the LuxR biosensor strain (Kviatkovski et al., 2015).

In the present study we describe the use of a LuxR-harboring whole-cell biosensor strain to specifically detect *P. aeruginosa* infections, via binding of 2-AA, in patients suffering from otitis externa.

2. Material and methods

2.1. Preparation of biosensor cells and 2-AA samples

E. coli/pSB401 (the biosensor strain) was grown with shaking (180 rpm) in Luria-Bertani (LB) broth supplemented with 20 μg/ml of tetracycline at 37 °C. Following overnight incubation, the cells were washed in 0.9% NaCl saline solution and diluted 100 fold in LB to obtain a concentration of approximately 10^6 cells/ml. Ten microliter of the biosensor cells were immobilized on a solid medium (described below) in the inner part of a 1.5 ml Eppendorf tube cap. Five hundred microliters of the analyzed sample, either 2-AA solution (Sigma-Aldrich, St. Luis, MO, USA) or pus sample (provided by the Edith Wolfson Hospital), suspended in Ringers lactate buffer (Hartmann's solution, Teva Medical; 0.2 g/l calcium chloride, 3.1 g/l lactic acid, 0.3 g/l potassium chloride and 6 g/l sodium chloride), were placed within the Eppendorf tube. In this setting there was no direct contact between the sample at the bottom of the Eppendorf tube and the biosensor strain in its cap, thus allowing activation only through air-borne substances. The tubes were closed and incubated for 18 h prior to measurement of the luminescence produced by the biosensor strain. The luminescence produced by the biosensor strain was measured either in a Photo Multiplier Tube (PMT) based plate reader or by our PMT-based compact biosensor device, as described below.

2.2. Long-term viability of the biosensor strain on different solid media

In order to evaluate the long-term stability of the biosensor strain we examined three types of solid media polymers for immobilizing the bacterial biosensor: water-agar, LB-agar and sodium alginate. For preparation of water agar-immobilized biosensor, overnight grown and washed cells were mixed 1:1 with 3% agar at 40 °C. Then, 250 μl of the mix were transferred to the cap of an Eppendorf where they were allowed to solidify. For preparation of LB-agar-immobilized biosensor, 10 μl of the cells were plated on 250 μl LB-agar matrix (1.5%), which was allowed to solidify within the cap of the Eppendorf tube. For preparation of alginate-immobilized biosensor, washed cells were mixed 1:1 with 3% sodium alginate solution in water. 250 μl of the mix were added to the cap of the Eppendorf. Further, 10 μl of 1 M $CaCl_2$ were added to the mix for solidification and encapsulation of the biosensor cells.

Following immobilization of the biosensor strain in each of the three different media, all the treatments were stored at 4 °C. The detection of 2-AA was examined using (i) 24 h old immobilized biosensor matrix, (ii) two week old immobilized biosensor matrix and (iii) five week old immobilized biosensor matrix. 2-AA solutions of 2, 10 or 100 nmol were added to the Eppendorfs with the different matrices, and were incubated overnight at 37 °C, as described above. Eppendorfs containing Ringers lactate buffer without 2-AA served as control. Following overnight incubation, the bioluminescence produced by the biosensor was evaluated by transferring the biosensor cells into a 96-well plate and measuring luminescence using a PMT-based infinite-F200 plate reader (Tecan Trading AG, Switzerland).

2.3. Optimal incubation temperature for 2-AA detection

For evaluating the optimal temperature for the assay, the biosensor strain was prepared as described above and immobilized on LB agar within the cap of the Eppendorf tube. 2-AA was added in amounts of 10 or 100 nmol to the Eppendorf tubes, which were transferred either to 30 °C or 37 °C. Following incubation of 18 h the biosensors were analyzed in the PMT plate reader as describe above.

2.4. Analysis of pus samples

Pus samples were collected from the external ear canal of 26 subjects clinically diagnosed as suffering from external otitis. All subjects provided informed written consent for their samples to be tested according to procedures that were approved by the Edith Wolfson Hospital Helsinki Committee. The subjects were of both sexes, with varied ages, from a few month infants to 85 years old seniors. All samples were analyzed for the presence of *P. aeruginosa* using three different methods as follows: A small portion of the pus sample was used for routine culture assays, which included the following growth mediums: blood agar, chocolate agar, Thioglycolate broth, Phenylethyl alcohol, Sabouraud, CHROMagar Orientation and MacConkey agar. The remaining pus sample was divided to two equal subsamples: one was subjected to the dynamic head-space analyses using GC–MS for 2-AA detection and the other was analyzed by the biosensor. Pus volumes varied between the different patients reaching a maximal volume of about 1 ml. In order to assure equal volumes in all analyses, lower amounts of collected pus samples were supplemented to a final volume of 1 ml using Ringers lactate buffer. For dynamic headspace analysis, 500 μl of the pus suspended in Ringers lactate buffer were placed in 20 mm headspace vials. The headspace vial was agitated and incubated at 60 °C for 10 min. The headspace was continuously collected for 1 h using 500 ml of Helium at 20 ml/min, unto a Tenax TA tube. The Tenax tube was desorbed in a Thermal desorption unit (Gerstel TDU) for 4 min at 21 °C in splitless mode and vapors subsequently trapped in a programmed temperature vaporizing (PTV) injector (Gerstel CIS4) that was kept in −70 °C. After desorption was complete, the PTV was heated at 12 °C/sec to 300 °C and was held there for 4 min. The GC Column was Restek Rxi-XLB medium polarity column, 30 × 250 × 0.25. The flow was 1.1 ml/min, oven program was 40 °C for 3 min then 12.5 °C/min to 300 °C for 3 min. Mass spectra acquisition was done in selective ion monitoring (SIM) mode, for masses 92.0;120.0;135.0.

The second subsample was examined with the biosensor strain for activation of the LuxR receptor by 2-AA as follows: 500 μl of the pus sample, suspended in Ringers lactate buffer, were transferred to an Eppendorf tube containing the biosensor strain immobilized on LB-agar in the inner part of the Eppendorf cap as described in Section 2.2. The volume of the analyzed samples was chosen to be 500 μl as this was the maximal amount of pus enabling equal partitioning to both GC–MS