Highly sensitive screening for antibiotic resistance using Parylene-matrix chip

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β-lactamase (EC 3.5.2.6) are an important family of enzymes that confer resistance to β -lactam antibiotics by catalyzing the hydrolysis of these antibiotics, as shown in Fig. 1. Some bacteria that can produce the β-lactamase have a resistance for β-lactam antibiotics. β-lactamase activity has been assayed using a number of techniques. However, most current assays are laborious and time-consuming. In this work, simple, rapid, and accurate assay was carried out using MALDI-TOF mass spectrometry. MALDI-TOF mass spectrometry has been used for the analysis of biomolecules with high molecular weights such as peptides, proteins, and nucleotides. MALDI-TOF MS has many advantages, such as easy sample preparation, low sample consumption, and so on. However, when organic matrix molecules are evaporated and ionized during the MALDI process, the mass peaks of the resulting fragmented matrix molecules are observed at very low mass-to-charge (m/z < 500) ratios. The Parylene-matrix chip was developed for the quantitative analysis of small molecules and great improvement of signal-to noise (S/N) ratio. The β-lactamase assay for the *E. coli* antibiotic susceptibility test was carried out using Parylene-matrix chip and MALDI-TOF mass spectrometry

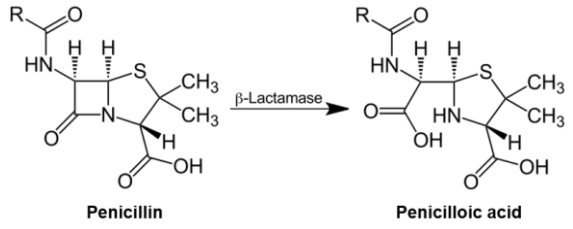

Figure 1. Hydrolysis of penicillin catalyzed by β-lactamase.

The Parylene-matrix chip was fabricated as describe below. As shown in Fig. 2, a matrix solution containing 2,5-dihydroxybenzoic acid (DHB) in acetonitrile with 0.1% trifluoroacetic

acid was dropped onto each sample spot of a stainless steel MALDI-TOF target plate. After drying the spotted matrix, a 50 nm of Parylene-N film was coated on the stainless steel target plate. The *E.coli* strain UT5600 (DE3), which lacks β-lactamase activity, and the penicillin-resistant variant UT5600 (DE3) pET-Z-18-3 with β-lactamase activity were grown in LB medium with carbenicillin.

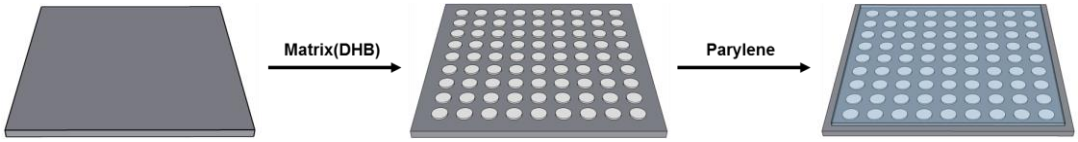

Figure 2. Fabrication of Parylene-matrix chip for the β-lactamase assay.

The Parylene-matrix chip was applied for the quantitative analysis of penicillin first. The signal-to-noise (S/N) ratio of penicillin with conventional matrix call DHB was estimated to be 9.36. When the Parylene-matrix chip was used to analyze penicillin, the signal-to-noise (S/N) of penicillin were greatly improved to 507.79 and 143.53 for $[PEN+H]^+$ and $[PEN+Na]^+$. The limit of detection of penicillin was estimated to be 0.25 μM (125 fmol/spot).

The Parylene-matrix chip was used in a β-lactamase assay. The assay measured the hydrolysis of penicillin into penicilloic acid with minimal interference of low molecular weight noise peaks. After hydrolysis of penicillin, the mass peaks of penicillin were observed as two different ions (m/z: $[PEN+H]^+$=335.1 and $[PEN+Na]^+$=357.8), whereas the mass peaks of penicilloic acid were observed as three different ions (m/z: $[PA+H]^+$=353.1, $[PA+Na]^+$=375.4, and $[PA-CO_2+H]^+$=309.1). The limit of detection for β-lactamase activity was estimated to be 20 μU/ml, as shown in Fig. 3. Penicillin-susceptible *E.coli* and penicillin-resistant *E.coli* were prepared and mixed with penicillin to validate the antibiotic susceptibility test. The penicillin-susceptible and penicillin-resistant *E.coli* strains showed different mass signal ratios at an *E.coli* concentration of 10^6 cells/ml, which corresponds to an absolute number of 1000 *E.coli* cells.

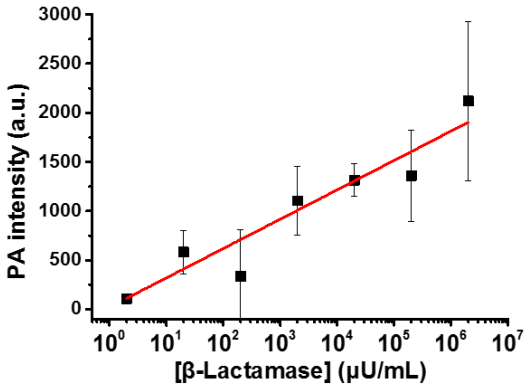

Figure 3. Quantitative analysis of the β-lactamase assay performed with the Parylene-matrix chip.