Electronic Supplementary Material (ESI) for RSC Advances.

Bacterial response towards soluble and immobilized AMP molecules revealed through global transcriptome analysis

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Experimental Details

Lasio-III which is naturally amidated in the C-terminus (VNWKKILGKIIKVVK-NH_2) and its N-terminus cysteine modified variant (CLasio-III) were chemically synthesized and procured from GL Biochem, Shanghai, China. All peptides employed in this study had a purity of >90%. The bacterial strain used in this study was *Escherichia coli* (ATCC8739).

Peptide immobilization

Substrate preparation: Silicon wafers (2.54 cm radius) were obtained from Latech, Singapore. The wafers were first washed by boiling with 0.05% SDS solution for 30 min followed by washing with copious amounts of (DI) water. The washed surfaces were then heated to 70^0C in basic piranha solution comprising ammonium hydroxide, hydrogen peroxide and water (1:1:5 v/v). The oxidised silicon surfaces were thoroughly washed with DI water and dried in a stream of nitrogen. The surfaces were then amino-silanized with 2% v/v amino propyl triethoxysilane (APTES) solution in anhydrous toluene for 1 h. The slides were cured at 100ºC for 1 h and washed with dry dimethyl formamide (DMF) before further treatment for peptide immobilization.

Spacer-mediated immobilization of CLasio-III on silanized surfaces: The amino-silanized surfaces prepared above were first PEGylated using Succinimidyl-([*N*-maleimidopropionamido]-polyethylene glycol ester (NHS-PEG-Mal) (Thermo Scientific Inc.). The NHS-PEG-Mal molecule consisted of two functional groups, i.e., NHS (N-hydroxy succinimide) and maleimide, which were separated by a PEG spacer arm. In this

study,the PEG spacer arms consisted of 24 ethylene oxide moieties and were designated as NHS-PEG(24)-Mal. 0.2 mg/ml NHS-PEG(24)-Mal solutions were prepared in dry DMF and allowed to react with two sets of amino-silanized surfaces for 4 h. The resulting surfaces were washed thoroughly with DMF followed by water and PBS. The PEGylated silicon surfaces produced were treated with CLasio-III in PBS at a concentration of 0.2 mg/ml.

Characterization of CLasio-III-immobilized surfaces: The different peptide immobilization steps were monitored using water contact angle measurements, X-Ray Photoelectron Spectroscopy (XPS) and ellipsometry as described in our earlier study. [1]

Study of bacteria response when treated with soluble and immobilized CLasio-III.

Exponentially growing *E.coli* cells (OD_{600} = 0.5) were used for all the experiments. For evaluating the activity of the peptide in solution form, 2 ml of the bacterial suspension was incubated with 3.4 µl of Lasioglossin-III solution (1 mg/ml in PBS) at 37 ºC for 30 min under shaking conditions. The control sample comprised an equal volume of PBS added to 2 ml bacterial suspension. To evaluate the activity of the immobilized peptide, the peptides were coated on silicon wafers prepared as per the method described above, while the PEG coated wafers (without any peptide) constituted the control surfaces. Each coated silicon wafer was placed in a 90 mm petri-dish containing 2 ml of the exponentially growing bacterial culture. The petri-dishes containing the coated wafers and bacterial cultures were incubated at 37 °C for 30 min under mild shaking conditions. Following incubation, the cells were harvested and washed with PBS buffer before mRNA isolation using the Qiagen RNAeasy kit, as per the manufacturer's protocol.

Microarray analysis

Microarray analysis of the extracted mRNA samples were carried out at Genomax Technologies (Singapore). 100 ng of total RNA was labelled with Low Input Quick Amp Labelling Kit, One-Color (Agilent, U.S.A.) following manufacturer's instructions. Briefly, 100 ng of total RNA was converted into double-stranded cDNA by priming with an oligodT primer containing the recognition site for 3-CTP labelled cRNA. 600 ng of labelled cRNA was hybridized on Agilent SurePrint G3 Human GE 8x60K Microarray for 17 h at 65 ºC, 10 rpm in Agilent hybridization oven. After hybridization, the microarray slide was washed in gene expression wash buffers before scanning on Agilent High Resolution microarray scanner (C-model). Raw signal data were extracted from the TIFF image with Agilent Feature Extraction Software (V10.7.1.1) and analysed using the Genespring software (Agilent, U.S.A) as described in an earlier study.[2] The Database for Annotation, Visualization and Integrated Discovery (DAVID) [version 6.7] tool was used for clustering the regulated genes with respect to their functional annotations,[3] where only the enrichment terms possessing less than 5% false discovery rate (FDR) were considered. Further functional analysis of the genes were done using different databases like the Regulon DB, EcoCyc, KEGG and PANTHER. The normalised gene expression values were fed to the MEV software for heat-map generation.

1. B. Mishra, A. Basu, R. Saravanan, L. Xiang, L. K. Yang and S. S. J. Leong, *RSC Advances*, 2013, 3, 9534-9543.
2. S. Basak and R. Jiang, *PloS one*, 2012, 7.
3. D. W. Huang, B. T. Sherman and R. A. Lempicki, *Nat. Protocols*, 2008, 4, 44-57.