

Facile and high-throughput assay of antimicrobial peptides with controlled fluorescence resonance energy transfer (FRET)

김영수, 차형준*

POSTECH

(hjcha@postech.ac.kr*)

Antimicrobial peptides generally kill bacteria through membrane permeabilization as an innate defense system as a promising alternative for conventional chemical antibiotics against multi-drug resistance bacteria. Here we describe a new preliminary assay system for high-throughput screening of antimicrobial peptides using pH-based FRET. This novel assay depends on the control of FRET efficiency with pH induced by instability of EYFP at a low pH. With antimicrobial peptide, the fluorescence ratio, Y/C, of ECFP-EYFP expressed in *E. coli* was decreased by lowering extracellular pH from 7.0 to 6.0 because released ECFP-EYFP from disrupted cells by antimicrobial peptide lost its yellow fluorescence, whereas Y/C ratio with BSA having no antimicrobial activity was maintained constantly due to the homeostasis of intracellular pH to about 7.0. The variation of ratio was reduced in direct proportion to antimicrobial activity. The assay showed more sensitive and accurate result with less quantity of antimicrobial peptides within 2 h compared to conventional method, and was also improved by introducing microplate fluorescence reading system for high-throughput assay.