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A facile, rapid and sensitive detection of MRSA using a CRISPR-mediated DNA FISH method, antibody-like dCas9/sgRNA complex

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Rapid and reliable diagnosis of methicillin-resistant Staphylococcus aureus (MRSA) is crucial for guiding effective patient treatment and preventing the spread of MRSA infections. Nonetheless, further simplification of MRSA detection procedures to shorten detection time and reduce labor relative to that of conventional methods remains a challenge. Here, we have demonstrated a Clustered regularly interspaced palindromic repeats (CRISPR)-mediated DNA-FISH method for the simple, rapid and highly sensitive detection of MRSA; this method uses CRISPR associated protein 9/single-guide RNA (dCas9/sgRNA) complex as a targeting material and SYBR green I (SG I) as a fluorescent probe. A dCas9/sgRNA-SG I based detection approach has advantages over monoclonal antibody in conventional immunoassay systems due to its ability to interact with the target gene in a sequence-specific manner. The detection limit of MRSA was as low as 10 cfu/ml and was found to be sufficient to effectively detect MRSA. Unlike conventional gene diagnosis methods in which PCR must be accompanied or genes are isolated an analyzed, the target gene can be detected within 30 min with high sensitivity without performing a gene separation step by using cell lysates. We showed that the fluorescence signal of the MRSA cell lysate was more than 10-fold higher than that of methicillin-susceptible *S. aureus* (MSSA). Importantly, the present approach can be applied to any target other than MRSA by simply changing the single-guide RNA (sgRNA) sequence. Because dCas9/sgRNA-SG I based detection approach has proved to be easy, fast sensitive, and cost-efficient, it can be applied directly at the point of care to detect various pathogens as well as MRSA in this study.

Biography

Kyonghye Guk obtained her bachelor's and master's degree in polymer nano science and technology from Chonbuk National University. Now She is studying doctor's course at UST. Her research efforts are on development of smart nanosystems for infectious disease diagnosis.

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