The Establishment of an Antibiotic Screening Method Based on an *in Vitro* Transcription and Translation System

Yao Wang[1], Shaoting Weng[1], Yiwen Liu[2], Sen Lin[3], Yaoyu Xu[4, *]

[1]School of Bioengineering and Food Science, Anyang Institute of Technology, Anyang, China

[2]Anyang Tumor Hospital, Anyang, China

[3]Anyang Kindstar Global Medical Laboratory Co., Ltd., Anyang, China

[4]School of Chemistry and Chemical Engineering, Anyang Normal University, Anyang, China

Email address:

liangtianer@163.com (Yaoyu Xu)

[*]Corresponding author

Abstract

The proliferation of antibiotic resistance among bacteria, attributed to genetic mutations and acquisition of exogenous genes, has been exacerbated by the extensive use of antibiotics. This has led to an urgent need for the development of novel antibiotics to maintain their therapeutic advantage. Our research group has established several in vitro transcription and translation systems, which have shown considerable potential for high-throughput antibiotic screening. We plan to develop additional systems based on Klebsiella pneumoniae and Staphylococcus aureus to identify antibiotics targeting a range of pathogens. We have successfully established a robust E. coli-based in vitro transcription and translation system that has enabled the stable expression of various proteins, including GFP, CD20, SARS-CoV-2 spike, membrane and envelope proteins. By incorporating known antibiotics such as chloramphenicol, streptomycin, spectinomycin, and orbifloxacin into this system, we have been able to assess their inhibitory effects on bacterial transcription and translation pathways using SDS-PAGE and fluorescence intensity. Extracts from various sources of Scutellaria baicalensis, Thymus, and Artemisia argyi, containing unknown compounds, were added to the system to evaluate their impact on GFP expression. Compound monomers and targets were confirmed through fishing experiments. Notably, the addition of transcription and translation inhibitors such as chloramphenicol, streptomycin, and spectinomycin at micromolar concentrations resulted in the absence of green fluorescence, whereas cell wall and membrane inhibitors like ampicillin and orbifloxacin at equivalent concentrations had no effect on fluorescence. The application of this method to the screening of antimicrobial components in Artemisia argyi extracts revealed that it contains active components that inhibit the pathway. Subsequent research will focus on the fractionation and detailed analysis of these mixtures to identify specific components and their targets. Ultimately, three previously unreported compounds with antibacterial activity were identified, along with their specific targets. Our team utilizes a well-established home-made in vitro transcription and translation system for the screening of active components in traditional Chinese medicine, combined with rapid chromatographic separation methods to quickly separate compound mixtures, achieving the identification of drug inhibition effects on specific pathways within 48 hours. Furthermore we aim to adapt this method to eukaryotic in vitro transcription and translation systems for the screening of anticancer drugs, thereby accelerating the process of drug discovery and target identification.

Keywords

In vitro Transcription and Translation, Drug Screening, Chinese Medicine, Antibiotic