

AGRICULTURE

Antimicrobial Assays: Comparison of Conventional and Fluorescence-Based Methods

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Antimicrobial assays are important tools to test and screen the inhibitory effects of myriad compounds against microorganisms before establishing their inhibitory spectra (broad vs. narrow). Knowledge of the inhibitory spectra of antimicrobial compounds before their application in the fields of agriculture, biotechnology, and medicine is crucial. Various conventional and contemporary methods are available, but they vary in their sensitivity and efficacy. In this study, our objective was to measure and compare the sensitivity and efficacy of an agar-based diffusion bioassay and a fluorescence-based assay for antimicrobial activity.

In the conventional antimicrobial bioassay, agar well diffusion, spot-on-lawn, and disc diffusion assays were performed against pathogenic *Bacillus cereus*, *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium. In the fluorescence-based assay, a cell-membrane-permeable, live-cell-staining, green fluorescent dye, carboxyfluorescein diacetate succinimidyl ester (cFDA-SE) was used to label the bacteria. The dye efflux from labeled bacteria was proportionally related to antimicrobial activity of the

compound. Antibiotics such as chloramphenicol (Chl), gentamicin (Gen), and oxytetracycline (Oxy) were tested on bacteria to compare the sensitivity and efficacy of both the methods. These three antibiotics were selected on the basis of their established broad-spectrum activity through different mechanisms of action. In this study, based on conventional and fluorescence bioassays, gentamicin revealed antibacterial activity against both gram-positive and gram-negative bacteria tested, while chloramphenicol was least effective, and oxytetracycline was highly inhibitory against *B. cereus* and *L. monocytogenes*. cFDA-SE-based fluorescence assay was sensitive, but the effects of antibiotic interaction with the fluorophore and strain-dependent esterase activity on the fluorescence needs to be accounted for and further investigated in order to optimize the protocol.

Research advisors Arun Bhunia and postgraduate Atul K. Singh write, "Developing a fundamental tool to determine the inhibitory spectrum of natural antimicrobial agents or antibiotics on bacterial pathogens is essential. A highly sensitive and quantitative method can accurately assess the antimicrobial effect on target pathogens to avoid generations of resistance to antibiotics, instilling a strong foundation for creative research in students."