A chromogenic TLC bioautographic assay for the rapid detection of β-lactamase inhibitors

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Résumé

β-Lactam antibiotics, including penicillin derivatives, cephalosporins, and carbapenems, are among the most widely used treatments for bacterial infections. They are frequently co-administered with β-lactamase inhibitors to prevent enzymatic degradation by resistant bacteria. However, the few inhibitors currently available share closely related chemical structures, and resistance to these compounds is increasingly reported (1). This issue has been identified by the World Health Organization as a priority public health threat, highlighting the urgent need for new β-lactamase inhibitors.
While natural products constitute a rich source of bioactive molecules, their exploration remains challenging due to the high chemical complexity of natural extracts. To facilitate the detection of β-lactamase inhibitors in complex mixtures, we developed a thin-layer chromatography (TLC) bioautographic assay, a method combining chromatographic separation with an in situ enzymatic reaction (2,3). The method uses nitrocefin, a chromogenic cephalosporin that changes from yellow to red upon hydrolysis by β-lactamases (4). A key advantage of nitrocefin is its sensitivity to all known β-lactamases produced by both Gram-positive and Gram-negative bacteria. Using this approach, natural β-lactamase inhibitors can be directly visualized on the TLC plate as clear zones against a pink background.

Validation with the reference inhibitor sulbactam and clavulanic acid demonstrated the reliability repeatability and sensitivity of the assay, with clear dose and time dependent inhibition. This simple and rapid TLC-based method was adapted to the identification of potential beta-lactamase inhibitors present in fungal extracts, constituting a tool for screening complex natural extracts and for the discovery of new beta-lactamase inhibitors, and can be combined with antibacterial direct bioautography to identify multitarget compounds, thereby accelerating the discovery of new strategies to combat multidrug-resistant bacterial pathogens.

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Mots-Clés: thin, layer chromatography, bioautography, enzymatic inhibition assay, beta, lactamase, nitrocefin, fungal extracts