

Title: DEVELOPMENT OF METHOD BASED ON PRODUCTION OF CELL-DENSITY-DEPENDENT GFP FOR THE DETECTION AND FUNCTIONAL SCREENING OF COMPOUNDS WITH ANTIBACTERIAL ACTIVITY

Authors: Araújo Jr, SD¹ and Krüger, RH¹

Institution: ¹UnB - University of Brasilia (Campus Universitário Darcy Ribeiro, Brasília - DF)

Abstract:

Several studies have shown that cloning and expression of enzymes and bioactive compounds by using the metagenomic approach is an alternative to functional exploration of yet uncultured microorganisms. The detection of microbial or isolated clones with antimicrobial activity can currently be performed by direct visualization of the phenotypic alteration of colony, as pigmentation and morphology, or inhibition halo formation when done overlay assays against microorganisms indicators. These strategies have identified metagenomic clones with antimicrobial activity, but showed, even using several hosts, low resolution and sensitivity, indicating a need for more robust screening methods to lower detection limits. Thus, to improve the possibility of detection and screening of genes with antibacterial activity from metagenomic clones and isolates microbial, or even from extracts and compounds from other sources, it was developed an antibacterial assay method named "Cell-Density-Dependent Expression" (CEDDEX). This method is based on fluorescence production, through the expression of *gfp* gene, due to the activation of molecules N-Acyl homoserine lactones (AHLs), that are directly related to cell density of the producing bacteria used in antibacterial assay. Thus, the CEDDEX method was based on the ability of a metagenomic clone (or any compound) with antibacterial activity to inhibit or retard the growth of a bacterial cell producing a compound which is directly related to cell density and that it was detectable and measurable. The CEDDEX method had a sensitivity at least 100x higher when compared to traditional methods employed to detect clones and compounds with antibacterial activity. This new methodology will be able to be used as a strategy to increase the sensitivity of screening clones with antibacterial activity from libraries metagenomic and/or microbial isolates, expanding significantly the potential for bioprospecting. Therefore, the use of CEDDEX method together with continuous exploration of functional microbial diversity has the potential to generate new chemical compounds and bioactive drugs as well as accelerate the discovery of new genes with antibacterial activity of biotechnological interest.

Keywords: Antibacterial assay, Bioactive compounds, Biosensor, Functional screening, Metagenomic

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