Bioluminescense method for high-throughput screening of compounds against *Mycobacterium tuberculosis*

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There has been an increase of *Mycobacterium tuberculosis* strains that are resistant to the current anti-TB agents, mainly through acquired resistance by therapeutic failure, This fact has underscored the need of a quick development of antimycobacterial drugs that are more effective than those currently in use. Moreover, new methodologies to determine the bactericidal activity of these compounds have been proposed. This study describes the use of bioluminescent strains of *Mycobacterium tuberculosis* H37Rv – ATCC 27974 and *Mycobacterium tuberculosis* Erdman ATCC 35801 both containing the plasmid pSMT1 constructed with *luxA* and *luxB* genes from *Vibrio harveyi* in a screening to evaluate the antimycobacterial activities of anti-TB agents. The standardization of the technique was performed using isoniazid and rifampicin, as a drug standard and the results of Minimal Inhibitory Concentration (MIC) were 0.03 μg/mL and 0.03 μg/mL, respectively. These values were totally compatible with those obtained with Microplate Alamar Blue Assay (MABA). The standardization of the bioluminescence measurement of intracellular antimycobacterial activity was performed using the J774 murine macrophage-like cell line infected with *Mycobacterium tuberculosis* Erdman containing the plasmid pSMT1 and rifampicin as a drug standard and the result of MIC was 0.16 μg/mL similar with those obtained with the technique of colony forming unit (CFU). A total of 32 compounds were evaluated, 19 crude plants extracts and 13 synthetic compounds and the results of Minimal Inhibitory Concentration were compared with those obtained with the MABA. 02 crude plants extracts and 02 synthetic compounds were evaluated and the results of MIC and percent of inhibition were compatible with those obtained with the CFU technique. The overall agreements between the MICs obtained by MABA and the results obtained with the luciferase reporter strain of *Mycobacterium tuberculosis* and with bioluminescence measurement of intracellular antimycobacterial activity and the CFU technique encourage the use of this recombinant mycobacteria in high-throughput screening of compounds against *Mycobacterium tuberculosis*.

*Tese está disponível na Bibliotecas
do Instituto de Química – UNESP Araraquara
e Instituto Adolfo Lutz.
e-mail: satodn@netsite.com.br

Intestinal carriage of yeasts by children in hospitalar setting

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At the last decades the nosocomial infections caused by yeasts raised significantly especially by *Candida* yeasts. The infections source can be endogen or exogenous, since spores of unicellular and multicellular are kept viable for months and several yeasts species are found in skin and mucosa of healthy people. In a saprophytic state yeasts are found in the human gastrointestinal tract but the relationship between the presence of these microorganisms and their pathology is associated with several facts such as: number, variety of sites colonized, effective use of antibiotics, associated infections caused by another microorganisms and mainly disturbance due to lack of immunity and metabolic. Yeasts in the gastrointestinal tract can be transmitted fecal-oral direct or indirectly from an individual to another. The transmission of a strain in a