determining minimum inhibitory concentrations (MICs)

stratus plate reader

introduction

The minimum inhibitory concentration (MIC) of a compound is defined as the lowest concentration at which it prevents the growth of a target microorganism such as a bacterium. An MIC value shows how sensitive the organism is to the compound being tested and it is used to select a drug that would be efficient in treating an infection. A drug with a low MIC value is considered more effective as less of it is required to treat an infection, which in turn helps to lower the toxicity of the drug to the person being treated for the infection and to reduce the chance of the infecting microorganism becoming drug resistance. The current study was focused on testing the effectiveness of two tuberculosis drugs (A and B). Tuberculosis is caused by the bacterium *Mycobacterium tuberculosis (Mtb)*. In 2019 there were 10 million new cases of tuberculosis (TB), and the disease caused 1.4 million deaths worldwide (1). Tuberculosis remains the most common cause of death from a single infectious pathogen and the drug resistant strains of *Mtb* pose serious problems in the efforts to lessen the TB burden (1). Therefore, there is an urgent need for the development of new and more effective TB drugs, which in turn calls for research on understanding the mechanisms that allow *Mtb* to be a successful human pathogen and to become resistant to currently available drugs. The reported experiment is part of a study that is focused on the above-mentioned topics and it employed *Mycobacterium (Mycolicibacterium) smegmatis (Msmeg)*, a non-pathogenic soil organism closely related to *Mtb*, as a model (2).

It employed two automated systems, Stratus, a small LED-based plate reader (Cerillo, Inc., Charlottesville, VA) and a Pipetting Robot (OT2, Opentrons Labworks Inc., Long Island City, NY) for determining the MICs of the above-mentioned TB drugs for a wild type strain of *Msmeg* (mc^2155) and a specific mutant of this organism.

materials and methods

The three M. smegmatis strains used were wildtype (mc^2155), mutant (with a deleted gene), and complemented (carrying a replicable plasmid allowing the expression of the wildtype version of the deleted gene). *M. smegmatis* strains were grown at 37 °C in Middlebrook 7H9 medium in a 96-well plate supplemented with glycerol and Tween 80 at final concentrations of 0.2% and 0.05%, respectively, and Drugs A and B at desired levels (3). Employing the OT2 Pipetting Robot, each well was filled with various volumes of autoclaved medium and drug solutions prepared in the medium, giving a combined volume 100 µl. Then to each well, 100 µl of a mid-logarithmic stage culture of an appropriate strain of *M. smegmatis*, diluted with the medium, was added. The plate with inoculated cultures was positioned in the Stratus growth chamber. Finally, the growth chamber was secured inside a shaking incubator (New Brunswick Scientific Co, Edison, NJ) that was maintained at 37 °C and shaken at 180 rpm for 24 hours. The data for the optical density of the culture at 600 nm as logged by the Stratus growth chamber was retrieved and analyzed by use of Microsoft Excel Version 16.56.

results and discussion

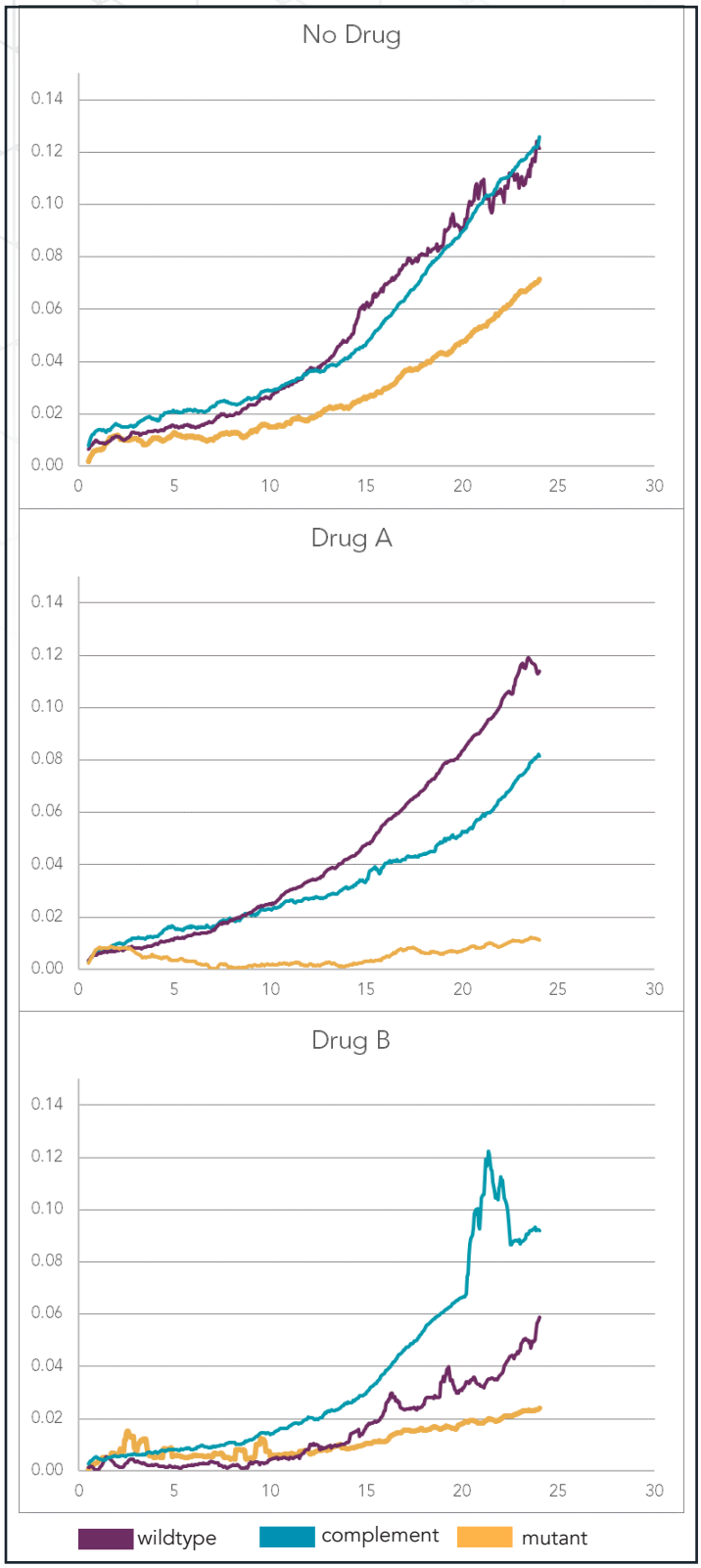

Figure 1. The top graph shows growth in the absence of drug. The middle graph shows the effect of Drug A at the highest concentration (0.1 µg/ml) on the growth. The bottom graph shows the effect of Drug B at the highest concentration (0.25 µg/ml) on the growth.

summary

The MIC for Drug A was less than that of Drug B for the mutant strain of *M. smegmatis*, and therefore, the former more effective in killing this strain.

references

1. Katoto P, Bulabula ANH, Sam-Agudu NA, Nachega JB, Tiberi S, McHugh TD, Abubakar I, Zumla A. 2021. Global Tuberculosis Report 2020 - Reflections on the Global TB burden, treatment and prevention efforts. Int J Infect Dis doi:10.1016/j.ijid.2021.02.107.
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3. Middlebrook G, Cohn ML. 1958. Bacteriology of tuberculosis: laboratory methods. Am J Public Health Nations Health 48:844-53.