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Test Report

Efficacy of a New JM Nanocomposite Material in Inhibiting *Mycobacterium tuberculosis*

Test Reagent
New JM nanocomposite material

Project Commissioner
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Abstract

Title: Efficacy of A New JM Nanocomposite Material in Inhibiting *Mycobacterium tuberculosis*

Experiment design: This project conducted laboratory tests on the efficacy of a JM nanomaterial in inhibiting *Mycobacterium tuberculosis* in a suspension. According to the Standard Operation Procedure of Communicable Diseases established by the Center for Disease Control of the Taiwan Ministry of Health and Welfare, the experiment inoculated *Mycobacterium tuberculosis* in suitable Middlebrook 7H11 agar plates and then incubated them at 36 °C with 10% CO_2 for 7 days until colonies formed. After cultivation, each mycobacterium formed a colony apparent to the naked eye. By calculating the number of colonies, the number of inoculated mycobacteria was inferred. In the experiment, the number of bacterial colonies in the control group was the number of inoculated mycobacteria, and the number of bacterial colonies in the experimental group was the number of bacteria remaining after being inhibited by the JM nanomaterial. The number of mycobacteria inhibited could be obtained by subtracting the number of colonies in the experimental group from the number in the control group number.

Test agent: New JM nanocomposite material

Agent provider: JM Material Technology, Inc., 5F-3, No. 40-2, Sec. 1, Minsheng N. Rd., Guishan Township, Taoyuan County

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Test Content

Experiment Materials

1. Tuberculosis bacteria source: *Mycobacterium tuberculosis* ATCC 27294 H37Rv strain
2. Middlebrook 7H11 agar plate: BioStar
3. Middlebrook 7H9 broth with OADC supplement: BioStar

Experimental Methods

1. Mycobacterium tuberculosis preparation
   (a) Inoculate the *Mycobacterium tuberculosis* into the Middlebrook 7H11 agar plate (7H11) and incubate at 36 °C with 10% CO_2 for 7 d until colonies form.
   (b) Scrape and remove the colonies; using Middlebrook 7H9 broth with OADC supplement (7H9), prepare a bacterial suspension to the McFarland no.1 standard.
   (c) Draw 100 uL of the tuberculosis suspension and add to 900 uL of 7H9; dilute at a ratio of 1:10.
   (d) Mix 100 uL of the above dilution to 900 uL of 7H9 and perform a 10-fold serial dilution.

Number	1	2	3	4	5	6
7H9（+ Trypsin）		1000	1000	1000	1000	1000
TB suspension	1100	0	0	0	0	0
Serial dilution	100	100	100	100	100	100 Discarding
Final volume	450	450	450	450	450	450
Final concentration	Former times	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}

   (e) Make two groups of the above preparation, labeling them as the control and experimental groups.

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2. Culture Test for the Inoculated 7H11 Agar Plates
    (a) Control Group
        i. Add 6.25 uL of sterile water to each tube.
        ii. Expose to UV at room temperature for 1 h.
        iii. Take 100 uL of the suspension and inoculate it into 7H11; then, use a sterile, disposable inoculation loop to spread it evenly over the entire culture medium.
        iv. Incubate at 36 °C with 10% CO_2 for 21 d of observation.
    (b) Experimental Group
        i. Add 6.25 uL of the JM nanomaterial to each tube.
        ii. Expose to UV at room temperature for 1 h.
        iii. Take 100 uL of the suspension and inoculate it into 7H11; then, use a sterile, disposable inoculation loop to spread it evenly over the entire culture medium.
        iv. Incubate at 36 °C with 10% CO_2 for 21 d of observation.
3. Interpretation
    (a) On each culture medium, six areas of 1 cm^2 area size were randomly observed for colony counts.
    (b) Formula for calculating the inhibitory efficacy of the JM material on *Mycobacterium tuberculosis*:
    Bacterial inhibition percentage = (Number of bacterial colonies in the control group - Number of bacterial colonies in the experimental group) / Number of bacterial colonies in the control group.

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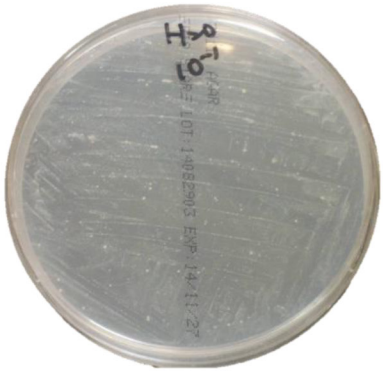
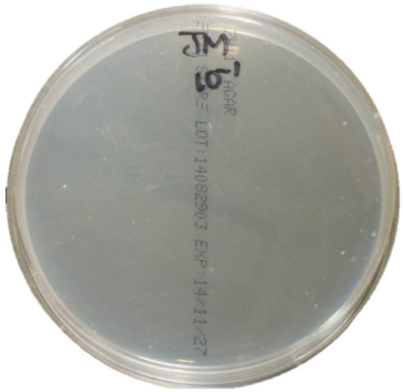
Test Results

1. The following images of the 10-fold dilution experiment are indicative of the overall test results. The white spots in the control group image are *Mycobacterium tuberculosis* colonies in the 7H11 culture medium. The experimental group image clearly shows that the number of bacterial colonies in the experimental group was vastly reduced, indicating that the JM nanomaterial effectively inhibited *Mycobacterium tuberculosis*.

	
Control group	**Experimental group**

2.Results of the random six areas are shown in the following table:

Concentration	Control group	Experimental group	Inhibitory efficacy
Original	>1000/cm^2	>1000/cm^2	Could not be calculated
10-fold dilution	>1000/cm^2	>1000/cm^2	Could not be calculated
10^2-fold dilution	>1000/cm^2	>1000/cm^2	Could not be calculated
10^3-fold dilution	105.3/cm^2	62.7/cm^2	40.5%
10^4-fold dilution	19.8/cm^2	7.7/cm^2	61.1%
10^5-fold dilution	2.6/cm^2	0.5/cm^2	80.8%

3.Calculation of the inhibitory efficacy of the JM nanomaterial on Mycobacterium tuberculosis: Substituting the results of the 10^5-fold dilution experiment (optimal) into the formula obtained an inhibitory efficacy of 80.8%.

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Conclusion

The experiment results show that the JM nanomaterial is able to inhibit tuberculosis when the *Mycobacterium tuberculosis* is diluted 10^5-fold. The percentage of *Mycobacterium tuberculosis* inhibition was 80.8%.