

Analysis summary of the effectiveness of MTR94 against bacteria and common mold

The enzyme, MTR94, was tested to determine its antimicrobial efficacy against a mixture of three common fungi and two common bacteria. Minimum Lethal Concentration (MLC) testing was the method used to determine the minimum concentration of enzyme required to kill the test microorganisms. MTR94 was found to be effective at killing the mixture of fungi and bacteria tested.

Procedure: All testing was performed in triplicate. The mean of the three results was considered the efficacy for that treatment. All microorganisms used were active cultures. For the first test, spores of *Aspergillus niger*, *Penicillium decumbens* and *Stachybotrys chartarum* (three common species of fungi) were mixed in sterile phosphate buffer to get a final concentration of 10^5 cfu/mL. Similarly, for the second and third tests, standard inoculums of *E. coli* and methicillin-resistant *Staphylococcus aureus* (MRSA) were prepared to get a final concentration of 10^5 cfu/mL.

MTR94 was prepared at concentrations of 20%, 40%, 60%, 80% and 100% in sterile purified water. For all of the above concentrations, 10-mL solutions were taken in three tubes for all tests. A control solution of 10-mL of 6% sodium hypochlorite solution (a common non-enzyme disinfectant) was also prepared in triplicate. A standard inoculum of 1-mL was added to each test tube. After a 24 hours contact period, 0.1-mL of each test was plated onto Malt Extract Agar (MEA) for the fungi and Tryptic Soy Agar (TSA) for the bacteria. Agar plates were incubated for a week at 25° C for fungi and 4 days at 35° C for bacteria. Following incubation, plates were examined for growth to determine the number of colony forming units (cfu). These would be spores or cells that were able to survive the enzyme treatment.

Conclusions/Observations: The following table summarizes the minimum concentrations of MTR94 that was able to achieve 100% efficacy to kill the test microorganisms in above experiments.

Test Organism	Contact Period	Concentration of MTR94	Viable Cells	Efficacy
Mixture of <i>Aspergillus niger</i> , <i>Penicillium decumbens</i> & <i>Stachybotrys chartarum</i>	24 hrs	100%	0	100%
MRSA	24 hrs	100%	0	100%
<i>E. coli</i> Detection limit: 10 cfu.	24 hrs	80%	0	100%


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