

# Lysozyme lab report

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Lab Report: Effects of Lysozyme Introduction: This report discusses an experiment that was done to demonstrate the effects of lysozyme on populations of Gram positive and Gram negative bacteria. Bacteria have a cell wall composed of peptidoglycan that gives the wall its strength. Gram negative bacteria have an extra component of lipopolysaccharide (LPS), that is stabilized with magnesium ions, to their cell wall that further protects them. When Gram positive bacteria are treated with lysozyme, the lysozyme breaks down the peptidoglycan, allowing the cell to rupture if it is in a hypotonic solution or change shape without rupturing if it is in an isotonic solution. The LPS of Gram negative bacteria protects the peptidoglycan from being affected by lysozyme. When Gram negative bacteria is treated with ethylene diamine tetraacetic acid (EDTA), a chelator, this destabilizes the LPS allowing it to become permeable. The objective of this experiment was to demonstrate that lysozyme will cause bacteria cell walls to rupture, causing a decrease in population, depending on the osmolarity of its surroundings. Methods: In this experiment three groups of samples were made. The first and second group contained Gram positive bacteria. The first group contained a population of *Micrococcus* that had been grown slowly and was still growing. The second group contained a population of *Micrococcus* that had been grown rapidly and was in the stationary phase. The third group contained a population of Gram negative bacteria. Each sample was placed in an HEPES buffer at a pH of 7.2 and concentration of 1.0 mM. This created a hypotonic solution. The first and second group contained three samples each. The first sample was a control and contained only the buffer and the *Micrococcus*. The second sample contained the buffer, the

Micrococcus, and the lysozyme. The third sample contained the buffer, the Micrococcus, the lysozyme, and KCl used to create an isotonic solution. The third group contained five samples. The first sample was a control and contained only the buffer and the Gram negative bacteria. The second sample contained the buffer, the Gram negative bacteria, and the lysozyme. The third sample contained the buffer, the Gram negative bacteria, the lysozyme, and EDTA. The fourth sample contained the buffer, the Gram negative bacteria, and EDTA. The fifth sample contained the buffer, the Gram negative bacteria, and a solution of KCl. Each sample was prepared with all the components except the lysozyme and an optical density reading was taken. This was used to determine the size of the population at 0 time. After this reading was taken the lysozyme was added to the appropriate samples and timing began. Optical density readings were taken at 2 minutes, 5 minutes, 10 minutes, 15 minutes and 20 minutes. These optical density readings were used to track the effects of the lysozyme on the activity of the bacteria. Results: After all the optical density readings were recorded, each reading was divided by the initial reading at 0 time to get the fraction of the initial optical density reading. This was used to show what percentage of the initial population remained at each recording. Gram positive bacteria Q represents the Gram positive bacteria sample in which only the lysozyme was added. R represents the Gram positive bacteria sample in which lysozyme was added in an isotonic solution. T represents the Gram positive bacteria sample in which only the lysozyme was added. U represents the Gram positive bacteria sample in which lysozyme was added in an isotonic solution. Gram negative bacteria B represents the Gram

negative bacteria sample in which only the lysozyme was added. C represents the Gram negative bacteria sample in which lysozyme and EDTA was added. (Note the y-axis begins at .089 to better illustrate the differences in sample populations.) This chart illustrates the differences of how lysozyme affects Gram positive and Gram negative bacteria.

Conclusions: This experiment demonstrated that when Gram positive bacteria was treated with lysozyme in a hypotonic solution the cells were lysed and populations declined. The lysozyme destroyed the peptidoglycan and allowed an inflow of fluid from the surroundings of the cells. When the Gram positive bacteria were in an isotonic solution, created by the addition of KCl, even though the lysozyme would have destroyed the peptidoglycan, there was no net inflow of fluid that would have caused the cells to lyse. The amount of decline in the Gram positive bacteria was dependant on whether the cells were growing or in a stationary phase. The cells that were still growing showed less of a decline than the cells in the stationary phase. Since the growing cells are still dividing and increasing more rapidly in number than the stationary cells, which are in a phase where there is no net increase in cell numbers, the effects of cell death are seen less dramatically. With the Gram negative bacteria, the expected results were that the cells treated with EDTA and lysozyme would have a greater decrease in population than the sample that was only treated with lysozyme. The EDTA destabilized the lipopolysaccharide layer allowing the lysozyme to reach the peptidoglycan layer and destroy it. This allowed osmosis to occur in the hypotonic solution, lysing the cells. From the graph of the results this is seen to be the case. Gram negative bacteria have an extra layer of protection that protects it

from its surroundings. This can be seen on the graph compared to the Gram positive bacteria. It can be seen that lysozyme has less of an effect on the Gram negative bacteria.