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Transient Bacteria Removal by Concentrated Sulfuric Acid for Cell Pollution

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Abstract

We found and recommended a method for rapid and effective sterilization by using concentrated sulfuric acid in cell culture.

Keywords: Sulfuric Acid; Cell Pollution

Introduction

Contamination of bacteria and fungi is a major problem in the process of cell culture, which will lead to waste of time and energy for researchers. In order to save the cells, traditional method of removing bacteria is to add antibiotics (such as penicillin, amphotericin B, nystatin, damicin, Tai Fu, etc.) in the culture medium, or to increase the dose of antibiotics and the times of drug use [1-5]. However, due to the toxicity of antibiotics and bacteria resistance and other problems, the process of bacteria removal is tedious and inefficient, often changes the cell phenotype - cell apoptosis even death, slow growth, bacterial and fungal contamination recurring. We found and recommended a method for rapid and effective sterilization by using concentrated sulfuric acid in cell culture.

Methodology

The method is suitable for contaminated adherent cell lines. In the 25 CM^2 coning culture bottle, add 25 uL concentrated Sulfuric acid, about one drop; the medium will instantly turn yellow and quickly fall off. Then a large amount of washing with PBS containing 10^x streptomycin. In the process of washing, 25 uL of concentrated sulfuric acid could be dripped into PBS one more time. It should be noted that PBS containing the acid is immersed in the walls of the culture bottle, then poured out, and then washed with a large amount of PBS without acid, so that the remaining acids must be removed. At last, the primary culture medium was added, and the cell mass was still well attached, the cells were clear and aseptic, and the liquid was changed once a day. Cell microorganism contamination detection kit was used to detect aseptic growth. We tested cell culture medium that containing the acid with a final pH of 1.62.

Results

In the process of cultivating the adherent cell line Min-6, the pollution of a bacillus was caused by a Bacillus dendrite, although a variety of antibiotics were used, the proliferation of the bacteria was not completely controlled (Figure 1). We tried to use concentrated sulfuric acid to remove bacteria and achieved unexpected results. Once this method is used, the pathogen can be completely eliminated without affecting cell characteristics (Figure 2). It has been widely applied to other cell lines, and has played a role in eradicating pathogenic bacteria. During use, we should pay attention to the following points: First, safety protection. Because of the corrosiveness of concentrated sulfuric acid, the operator should wear gloves masks, and do not let them splash on the skin and other metal instruments such as operation panel. Second, the action is quick. After adding acid, the medium should be immediately removed, so as not to damage the cells. Third, the rinse is quick and thorough. To prepare sufficient PBS solution

containing 10^x streptomycin, the acid PBS should be quickly immersed into each wall of the culture bottle to prevent bacterial residue. Then clean the residual acid with the non-acid PBS. Fourth, frequently change the medium.

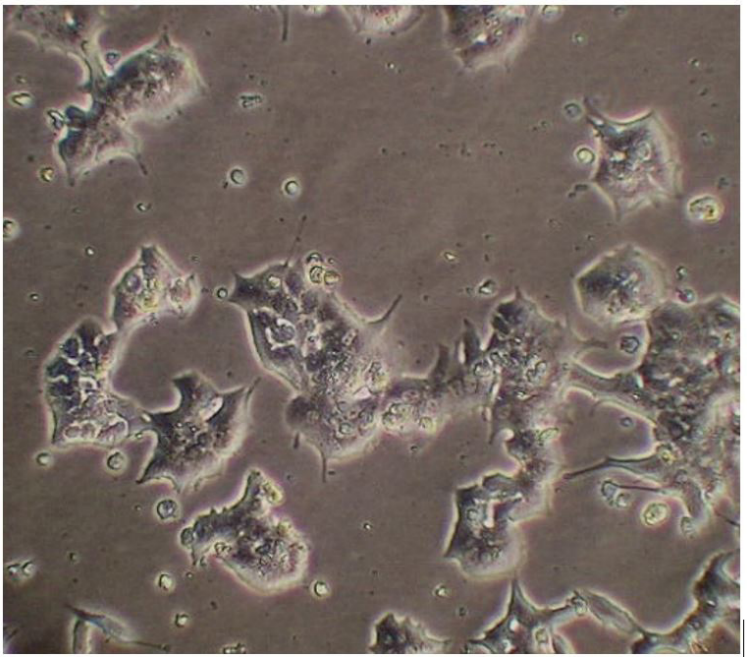

Figure 1: Min-6 cell bacterial infection: bright spot is bacteria

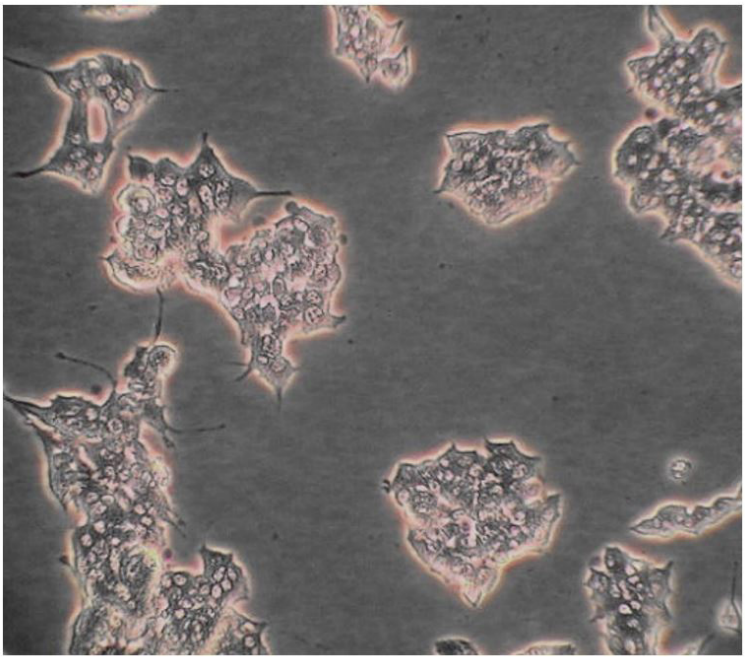

Figure 2: 3 days after acid treatment: no bacterial discovery

Discussion

Concentrated sulfuric acid has served in the field of cell culture and potassium dichromate and distilled water to prepare a cleaning solution for the pickling of glassware. Its strong oxidation can remove trace impurities that cannot be washed away. In addition, low pH can inhibit the growth of bacteria, and lactic acid, acetic acid, ethanol and other organic acids have been demonstrated to have a sterilizing function [6-8]. Therefore, as a non-traditional sterilization method, concentrated sulfuric acid has the function of rapidly cleaning the cell culture containers. Just its strong oxidizing corrosiveness, could instantly kill all microorganisms including bacteria and fungi. We have utilized its killing and washing ability for the first time in the process of removing bacteria from contaminated cells, and completely cleared the growth of bacteria and fungi. It is inevitable that the treated cells will cause some losses, but all the remaining cells are vigorous and require nutritional support. Containers after washing should be thoroughly cleaned to prevent residual acids from affecting cell growth. It is advisable to change the liquid once a day. This method can kill

pathogenic bacteria instantaneously and efficiently, and can be implemented to clean up the pollution of most pathogenic bacteria. It is simple to operate and does not change the characters of cell, which could be popularized in cell culture.

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