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Short Communication
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Using Sand Particles for the Disruption of Cell Walls of Gram-Positive Bacteria and Mycobacteria

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Short Communication

In the last decade, DNA-based molecular methods have come to the fore for the sensitive and rapid diagnosis of infectious agents [1], with the polymerase chain reaction (PCR) being particularly useful in this regard [2,3]. Obtaining sufficient pure DNA or RNA is important for PCR protocols. Cell wall disruption is the first and one of the most critical steps affecting yield and quality of isolated DNA and RNA.

For DNA isolation from gram-negative bacteria, different DNA extraction protocols have been used successfully. These include boiling, phenol-chloroform, proteinase K, lysozyme, and guanidine thiocyanate treatment followed by ethanol precipitation [1-3].

However, DNA or RNA isolation from gram-positive bacteria and *Mycobacterium* is extremely difficult. This is because Gram-positive bacteria have a thicker peptidoglycan layer while the cell walls of mycobacteria genus include complex glycolipids. Therefore, complete or partial removal of the complex cell wall structures of this microorganism are necessary to obtain sufficient DNA or RNA [4-6]. The bacterial cell wall disruption methods are usually mechanical or enzymatic. Mechanical methods include homogenization with a dounce or a mechanical homogenizer vortexing, sonication, French press, glass beads [1,7]. Enzymatic methods include using lysostaphin to form spheroplasts for the gram-positive bacteria *Staphylococcus* genus or using a chemical like cetyltrimethyl ammonium bromide for the *Mycobacterium* genus [5,6].

Recently, we published an article describing the use of sand particles for mechanical elimination of the cell wall without any need for chemicals [8]. Sand is a naturally occurring granular material composed of finely divided rock and mineral particles. We thought using sand for cell wall disruption would be more effective than glass since sand's surface is rougher and stronger than that of glass beads. Sand is also easy to find from the seaside or river banks. Assuming that the edge structure of the sand particles from stream banks is sharper than that of seaside sand because there is less friction on stream banks. We obtained stream bank sand which, we sieved to 0.5-3 mm in size. Before applying to DNA extraction, we washed the sand with ddH_2O to eliminate all dirt and dust without losing the small particles and then sterilized by autoclaving (Figure 1). To use in RNA extraction, the sand was washed with ddH_2O, incubated for 30 minutes with 10% HCl, and then autoclaved.

To determine if sand offers an effective method for DNA extraction, we mixed a loop of *Staphylococcus aureus* strains in 100 µl TrisEDTA buffer with 100 mg sand. We then vortexed this mixture at maximum speed for 3-5 minutes before treating it with proteinase K and phenol-chloroform. Finally, we followed the ethanol precipitation protocol to obtain the DNA. To compare this method to others, we incubated for one hour the same quantity of bacteria used in the sand method with lysostaphin before conducting proteinase K DNA extraction as in the sand method. We then ran the DNA samples obtained from MRSA using lysostaphin and from MRSA using sand in agarose gel electrophoresis, measuring DNA quantity and purity with a spectrophotometer. This showed that the two extraction methods produced similar quantities and purities of extracted DNA. We also evaluated the quality of the obtained DNA using PCR with specific primers. PCR, which worked effectively with DNA obtained, using the sand method, did not reveal any presence of DNA inhibitors.

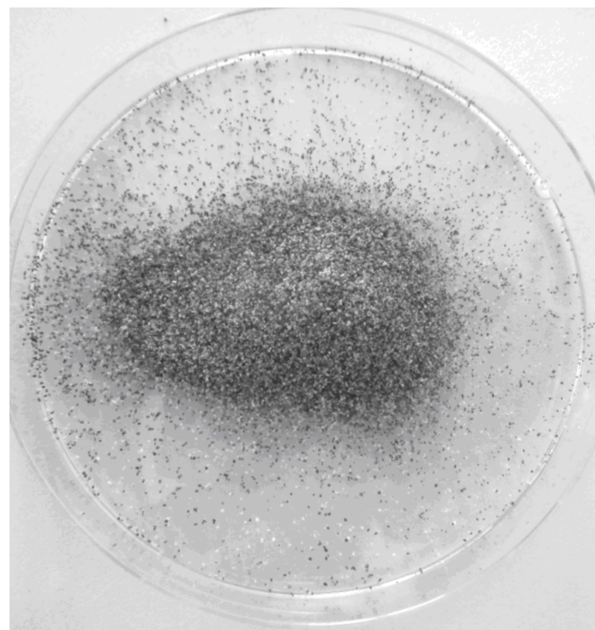

Figure 1: Sand was prepared as described in the text.

To obtain RNA from *M. Tuberculosis*, we dissolved the bacteria in 20 µl Tris-EDTA buffer with 100 mg sand. We vortexed the mixture at maximum speed for 5 minutes before following the standard RNA extraction protocol using guanidinium thiocyanate-phenol-chloroform (GTPC). To compare using sand to the other methods, we applied the GTPC protocol to the same quantity of mycobacteria without sand treatment. We obtained RNA efficiently from *Mycobacterium* strains using the sand method, whereas we could not obtain RNA from these strains using the GTPC method. To evaluate the quality of the RNA obtained by the sand method, we used cDNA synthesis followed by a test of PCR efficiency using primers specific to

M. tuberculosis genes. We found that the RNA that we obtained with the sand method was also usable for both cDNA synthesis and PCR using synthesized cDNA.

There are studies describing the usage of silica and zirconium particles in addition to the glass beads to disrupt bacteria cell walls [9]. Especially silica, both particles bind the DNA with high affinity. Although we didn't test this, we predict that extraction protocol using these two materials yields smaller amounts of DNA then using sand. In addition, silica and zirconium need to be treated with other chemicals before applying to DNA extraction.

As described above there are different mechanical and enzymatic methods. However, these not only require different equipment or enzymes but also take considerable time to complete cell wall disruption. In contrast, the sand method works effectively and efficiently yields sufficient quantities of pure DNA and RNA from bacteria with rigid cell walls. Using sand has various important advantages. In particular, pre-prepared sand can be used or stored almost indefinitely in the laboratory. It also decreases the time for DNA-RNA extraction. In conclusion, using the sand instead of relatively expensive equipment or lysostaphin and other chemicals decreases the cost and time required for DNA-RNA extraction.

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