Toxic buffers used in microbial sample preservation for SEM can be replaced by PBS

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The structural preservation of uncultivated, oxygen-sensitive microorganisms for imaging via scanning electron microscopy (SEM), often relies on toxic buffers like cacodylate. As such, studies on certain archaea named Candidatus Altiarchaeum which can dominate deep biosphere ecosystems by forming biofilms using hook-shaped cell appendages (hami), currently heavily rely on cacodylate buffers. However, the twelve principles of green chemistry state that researchers should strive to minimize the use of toxic substances and ideally replace them entirely with non-toxic alternatives. Following these principles, we here tested the influence of alternative, less toxic buffers (phosphate-buffered saline (PBS) and PHEM (PIPES-HEPES-EGTA-MgCl2)) for the preservation of Ca. Altiarchaeum hamiconexum for SEM and compared them with preservation using cacodylate for subsequent imaging.

Fixation of Ca. Altiarchaeum hamiconexum was conducted immediately after the collection of the biofilm from a cold sulfidic spring located in Regensburg (Bavaria). Samples were preserved using 4% formaldehyde final concentration in cacodylate, PHEM or PBS, respectively. For SEM preparation, 2.5% glutaraldehyde in cacodylate, PHEM or PBS, were used, after which samples were rinsed four times with the corresponding buffer and post fixated with 1% osmium tetroxide in cacodylate, PHEM or PBS. A dehydration gradient was then applied with 10%, 20%, 40%, 60%, 80% and 100% acetone. Quality of preservation was examined using SEM after critical point drying and sputter coating with platinum/palladium. The so prepared archaeal cells were analyzed in comparative manner by experienced scientists used to investigate Ca. Altiarchaeum hamiconexum ultrastructure prepared with cacodylate buffer.

The preservation with cacodylate and PBS revealed no noticeable differences. The hami displayed a high degree of preservation and no visible difference in the thickness and length of those structures. However, all cells, independent of the preservation buffer used, showed deformations. The samples preserved in PHEM exhibited the least preservation of the hami, with many cells showing complete absence of appendages.

To reduce risks for researchers and the environment, the use of toxic substances in laboratory conditions should be avoided if less dangerous alternatives exist. In this study, no visible differences were observed for the quality of hami preservation between toxic buffer cacodylate and PBS. PBS can effectively replace cacodylate for preserving the ultrastructure of hami of Ca. Altiarchaeum hamiconexum for the purposes of high-resolution microscopy.

Keywords:

Sanple preparation, Microbiology, Buffer replacement