Biomeme

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Assessment of *Pseudomonas aeruginosa* deactivation by Biomeme Lysis Buffers

INTRODUCTION

Four Biomeme lysis buffers were evaluated for their ability to inactivate *Pseudomonas aeruginosa (P. aeruginosa)* ATCC 10145 by following a 10-minute exposure at room temperature and 60℃.

MATERIALS AND METHODS

Bacterial Strain

P. aeruginosa ATCC 10145 was obtained from the American Type Culture Collection (ATCC) and propagated as recommended in Trypticase Soy Broth (TSB).

Propagation and Growth of *P. aeruginosa*

P. aeruginosa was received as a lyophilized powder and was resuspended in Nutrient Broth. A glycerol stock was prepared and frozen at -80℃. To obtain a culture for use in the assay a loop of the frozen culture was streaked onto a plate of trypticase soy agar with defibrinated sheep blood (TSA/5% SB) and incubated under aerobic conditions at 37℃ for 24 hours. From this plate, a single colony was used to inoculate a tube of Trypticase Soy Broth (TSB) and this culture was incubated for approximately 16 hours at 37℃. Following the incubation, the culture was adjusted to the appropriate optical density for use in the assay (1 x 10^8 CFU/mL).

Assessment of *P. aeruginosa* (ATCC 10145) by Biomeme Lysis Buffers

Two hundred microliters (200 µL) of *P. aeruginosa* at 1 x 10^8 CFU/mL was added to each inactivating agent in the volume indicated in **Table 1** in a 15 mL conical tube. The samples were incubated at room temperature or 60℃ for 10 minutes. It should be noted that for the 60℃ samples, the incubation time was not initiated until the sample reached 60℃ internally. Following the incubation, the reaction was stopped by diluting the sample 1:10 with cold TSB. Each sample was then serially diluted eight times in ten-fold increments. Fifty microliters of each dilution for each sample was plated in duplicate on TSB/5% SB plates to determine the CFU/mL of each sample. An untreated control for was included at each bacterial concentration for comparison.

Table 1: Assay Conditions for Inactivating Agents

Inactivating Agent	Volume of Inactivating Agent (µL)	Volume of Bacteria (µL)	Final In-Well Concentration of Bacteria (CFU/mL)
DNA BLB Mixture 1	1,000	200	1×10^7
DNA BLB Mixture 2	200	200	5×10^7
DNA BLB Mixture 3	400	200	3.3×10^7
RNA BLB	1,000	200	1×10^7

RESULTS

Four inactivating agents were incubated with *P. aeruginosa* ATCC 10145 for 10 minutes at room temperature or at 60℃. The untreated control samples at room temperature had bacterial densities ranging from 1.4×10^7 to 3.6×10^7 CFU/mL. Exposure of the bacteria to each of inactivation samples resulted in no bacterial growth at room temperature. When exposed to 60℃, there was no bacterial growth with treated or untreated samples. Data are summarized in **Figure 1**. In summary: four inactivating agents were incubated with *P. aeruginosa* ATCC 10145 for 10 minutes at room temperature and 60℃. Each agent was able to inactivate the bacteria at room temperature.

Figure 1: Assessment of *P. aeruginosa* (ATCC 10145) by Biomeme Lysis Buffers