Report:

Testing the effect of StaBiotic cleaning products on viability of *Staphylococcus aureus* and *Clostridium difficile*.

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Report generated for BactaClean-Envirosystems (UK)

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RESULTS GENERATED.

(DEFINITION: For the PureBiotics® product tested, the acronym PIP refers to Probiotics In Progress, which is part of the ongoing methodology of how the probiotic base of PureBiotics® work): PIP-cleaning solutions were tested for their effect upon *Staphylococcus aureus* using the Meticillin-resistant strain MRSA252, a hospital epidemic strain isolated in the UK. In addition the solutions were tested for efficacy against *Clostridium difficile* MHCR strains 107 and 029. These isolates are two common clones causing epidemic outbreaks in UK hospitals.

EFFECT UPON *S. AUREUS*

S. aureus MRSA 252 was cultured in BHI broth (Lab M) from stored frozen cultures and grown overnight at 37°C. Cells were diluted in water or water containing 6.5% BHI to approx. 10^8 cfu per ml before addition of PIP cleaning solution to 1% (v/v) (Chrisal). At time points 0, 4 and 24 h samples were removed and serially diluted before plating on BHI agar. Viability was assessed after overnight growth at 37°C and the effect of PIP was plotted. A representative dataset for 0 and 24 h time points is shown below (figure 1) and is typical of triplicate experiments. No difference was observed between PIP floor cleaning solution and sanitary solution under the conditions tested (data not shown). Typically both cleaning solutions reduced viability around 10-fold over a 24 h period, producing a 2-3 fold reduction in viability at 4 h.

PIP cleaning solution was also tested on desiccated cells of *S. aureus* on sterile plastic surfaces after drying at 37°C and maintained at 25°C overnight. The aim of this was to mimic dried cells of *S. aureus* in a hospital ward. Drying reduced viability of *S. aureus* markedly; the addition of floor cleaning solution produced a similar reduction in viability of approx 10-fold to that observed in the liquid assays detailed above and shown in figure 1.

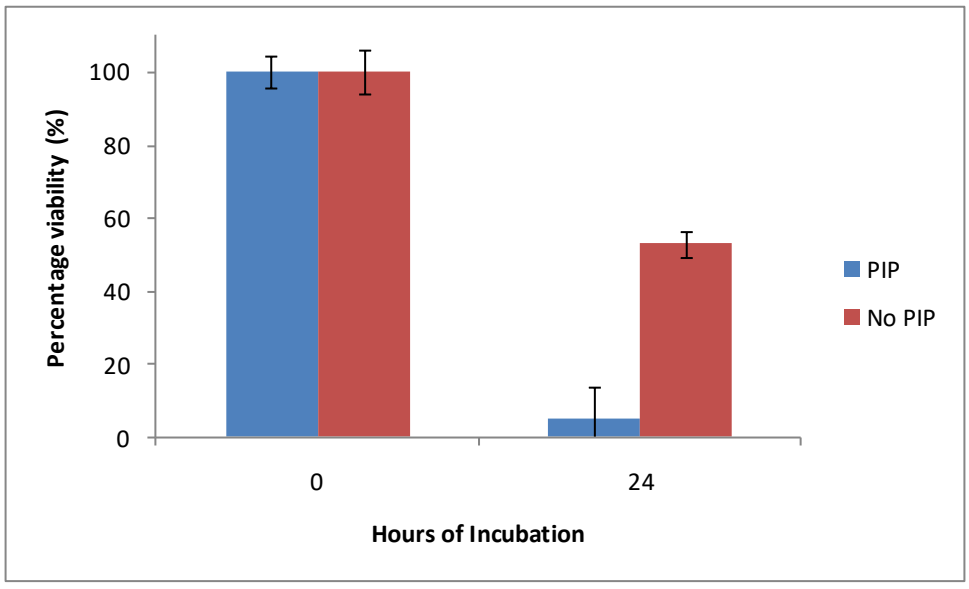

Figure 1: Effect of PIP solution on viability of *S. aureus* MRSA 252. Viability tested using 1% (v/v) floor cleaning solution upon overnight culture cells. Error bars represent SE of mean from 4 separate samples.

CONCLUSION:

Cleaning solutions diluted to 1% (v/v) reduce viability of *S. aureus* MRSA252 by approximately 10-fold after 24 h incubation compared to an untreated control.

EFFECT UPON *C. DIFFICILE*

Hospital outbreak strains of *C. difficile* were obtained from Royal Liverpool and Broadgreen University Hospital Trust. This study used MHCR109 and MHCR027. These isolates were cultured on fastidious anaerobe agar supplemented with 10%(v/v) blood in anaerobic containers at 37°C for 72 h to ensure the bacteria had sporulated. Spores were scraped from agar plates and washed in sterile water. Oil immersion microscopy revealed preparations were >90% spores. The spores were not heat treated prior to the assays. PIP cleaning solutions were tested for their effect upon the spores using the floor cleaning solution at 1% (v/v) in water with approx 10^5 cfu per ml in an aerobic assay. Samples were removed at 0, 4 and 24 h and serially diluted to calculate viable cells counts after overnight growth on FAA under anaerobic conditions at 37°C (figure 2).

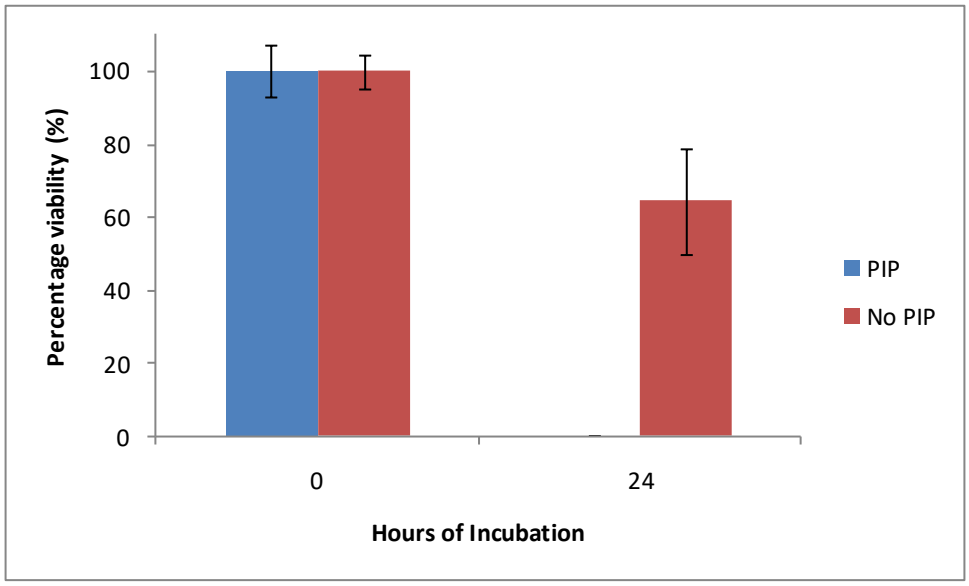

Figure 2: Representative experiment showing the effect of PIP floor cleaning solution (1% v/v) on survival of *C. difficile* spores of MHCR 109. Error bars represent standard error of the mean generated from quadruplicate samples. Experiments were repeated in triplicate.

CONCLUSION:

PIP floor cleaning solution diluted to 1% (v/v) markedly reduces viability of C. difficile compared to the untreated control, as judged by viable counts of treated spore suspensions after 24 h of incubation under the assay conditions tested here. The proposed effect of the enzymes in the product is to induce germination of *C. difficile*, which will be deleterious to the survival of this anaerobe in the aerobic conditions of the assay. The results obtained here are entirely consistent with this claim given the observed reduction in viability.