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Removal of bacterial biofilm from Escherichia coli by synergistic action of serine proteases and biosurfactant.

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Abstract
According to the Centers for Sterilization of Dental-Medical-Hospital Materials, the development of effective cleaning products in the removal of microbial load has been of the utmost importance. The addition of enzymes to these products promotes a beneficial effect, but different effects have been observed depending on the biofilm and the enzymes evaluated. The use of biosurfactants in therapeutic applications aims to inhibit bacterial adhesion and the formation of biofilms. Thus, the objective of this work was to evaluate the efficiency of these cleaning agents containing enzymes of the class of proteases and biossurfactant surfactin in the removal of bacterial biofilm of Escherichia coli under different concentration and time conditions.

Key words:
Proteases, biossurfactant, bacterial biofilm.

Introduction

Removal of dirtiness and bacterial biofilms from medical devices has been a major health care challenge to avoid hospital infections and high costs for the health care system[1]. It is important to stress that the use of more modern and complex but delicate medical devices and instruments can not be reprocessed with corrosive chemicals and high temperatures. In this way, softer solutions, such as enzymatic detergents and biossurfactants, must be carefully developed to ensure effective performance. This work describes the use of cleaning agents containing serine proteases and surfactin in order to remove the bacterial biofilm of *E. coli*.

Results and Discussion

The biofilm of *Escherichia coli* ATCC 35218 was grown in BHI medium for 24h at 37°C in 96-well plates for further analysis of biofilm removal. Two enzymes of the class of serine proteases (E1 and E2) at concentrations of 0.5% and 5.0% and surfactin at concentrations of 0.1% and 0.5%, diluted in detergent base were evaluated. 0.25% SDS and 0.9% NaCl were used as positive and negative controls, respectively. Violet crystal and tetrazolium salt were used to detect residual biomass and cell viability, respectively.

It was observed that E1 and E2 removed 65% and 55% of biomass in 0.5h, respectively, regardless of the dilution of the enzyme used. However, the exposure time increases biofilm removal to 90% (in 2.0h). Independently of the enzyme dilution E1 and E2 promoted 85% and 95% of cell death, respectively. However, no increase in the cell death was observed for the time of exposure of the biofilm to the proteases individually.

Surfactin at 0.1% concentration removed 38% of the total biomass in 0.5h and 46%, in 2h. At a concentration of 0.5%, 58% of biomass was removed in 2h. At the same surfactin concentrations, a cell death of 20% was observed, independently of the enzyme dilution and time exposure.

The joint action of the two proteases promoted the removal of 86% of biomass and a cell death of 89%. When 0.5% surfactin is added to the cleaning agent containing the proteases at 5% concentration, 90% the biomass removal and cell death are observed in 0,5h.

Our results showed that surfactin alone promoted biomass removal but was not efficient as bacteriostatic agent in the cell death. The use of the E1 and E2 proteases together was able to increase the biomass removal and the cell death. The addition of surfactin to the cleaning agent containing the proteases was able to promote greater biomass removal and cell death in the shortest time. Both the effects biomass removal and cell death depended on the enzyme and surfactin dilution and the time exposure.

Conclusions

The best conditions to use the cleaning agent to remove the biomass and to kill the cells were obtained from the synergistic actions of the two serine proteases and the biossurfactant surfactin. The use of enzymatic detergent as a cleaning product to remove microbial load from medical devices and instruments is hardly dependent on an efficient previous study of enzyme dilution and time exposure.

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