

Preliminary characterization of a novel antimicrobial surface coating against significant pathogens

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Introduction and objective

The survival and spread of resistant foodborne and nosocomial-associated bacteria through high-touch surfaces is not always prevented by the employment of cleaning protocols. Antimicrobial surface coatings surge from the need to eradicate pathogenic bacteria and prevent future infections and even outbreaks.

This study aimed to characterize a novel QAC-based coating in terms of cytotoxicity, kinetics, and durability and to determine its ability to inhibit important health-associated pathogens on different surface materials (polyvinyl chloride, glass, and stainless steel).

Methods

Antimicrobial activity and kinetics

Antimicrobial surface coating efficacy, killing contact time and durability



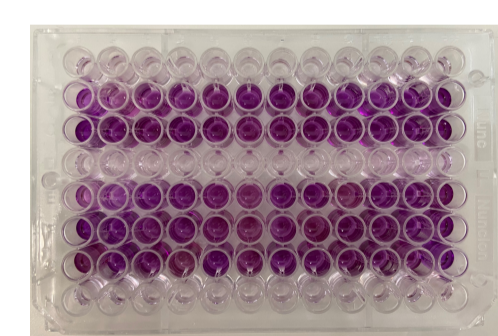
Escherichia coli ATCC 25922
Acinetobacter baumannii ESB260
Listeria monocytogenes Scott A

polyvinyl chloride (PVC)
glass
stainless steel

ISO 22196:2011

Antimicrobial coating cytotoxicity

Cytotoxicity of the antimicrobial surface coating was assessed through MTT assay



Human colorectal adenocarcinoma cells (trace concentrations)

References

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- Lea T. (2015) Caco-2 Cell Line. In: *Verhoeckx K. et al. (eds) The Impact of Food Bioactives on Health*. Springer; Cham. https://doi.org/10.1007/978-3-319-16104-4_10

Results and discussion

Antimicrobial activity, kinetics and durability

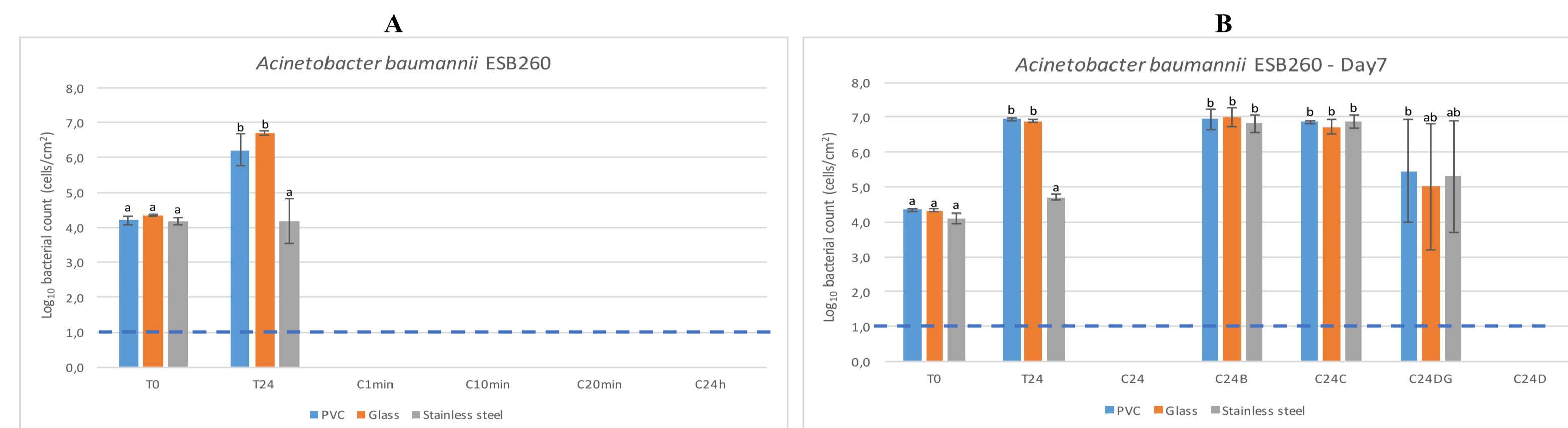


Figure 1. A) Contact killing time for *A. baumannii* ESB260. Recovery of bacteria immediately after inoculation (T0); after 24h incubation (T24) on untreated surfaces; Recovery of bacteria immediately after inoculation (C1min); after 10 minutes (C10min), 20 minutes (C20min) and 24h incubation (C24h) on each treated surface. **B)** Antimicrobial activity of the compound on day 7 for *A. baumannii* ESB260. Recovery of bacteria immediately after inoculation (T0); after 24h incubation (T24) on untreated surface; after 24h incubation (C24B) on treated surface cleaned with bleach; 24h incubation (C24C) on treated surface cleaned with damp cloth; 24h incubation (C24DG) on treated surface cleaned with commercial degreaser and 24h incubation (C24D) on treated surface cleaned with commercial disinfectant. The results are means based on data from three replicates and standard deviations are indicated by error bars. Equivalent lower case letters mean no significant differences between each condition ($p>0.05$). The dotted line means that the isolate was reduced to values below the detection limit of the incorporation technique.

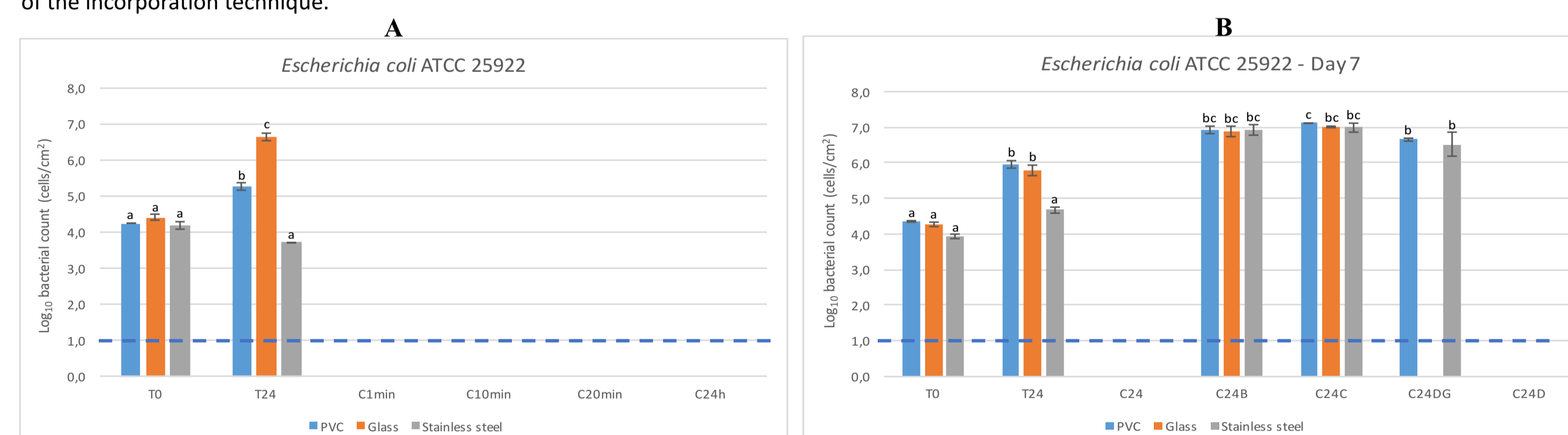


Figure 2. A) Contact killing time for *E. coli* ATCC 25922. Recovery of bacteria immediately after inoculation (T0); after 24h incubation (T24) on untreated surfaces; Recovery of bacteria immediately after inoculation (C1min); after 10 minutes (C10min), 20 minutes (C20min) and 24h incubation (C24h) on each treated surface. **B)** Antimicrobial activity of the compound on day 7 for *E. coli* ATCC 25922. Recovery of bacteria immediately after inoculation (T0); after 24h incubation (T24) on untreated surface; after 24h incubation (C24B) on treated surface cleaned with bleach; 24h incubation (C24C) on treated surface cleaned with damp cloth; 24h incubation (C24DG) on treated surface cleaned with commercial degreaser and 24h incubation (C24D) on treated surface cleaned with commercial disinfectant. The results are means based on data from three replicates and standard deviations are indicated by error bars. Equivalent lower case letters mean no significant differences between each condition ($p>0.05$). The dotted line means that the isolate was reduced to values below the detection limit of the incorporation technique.

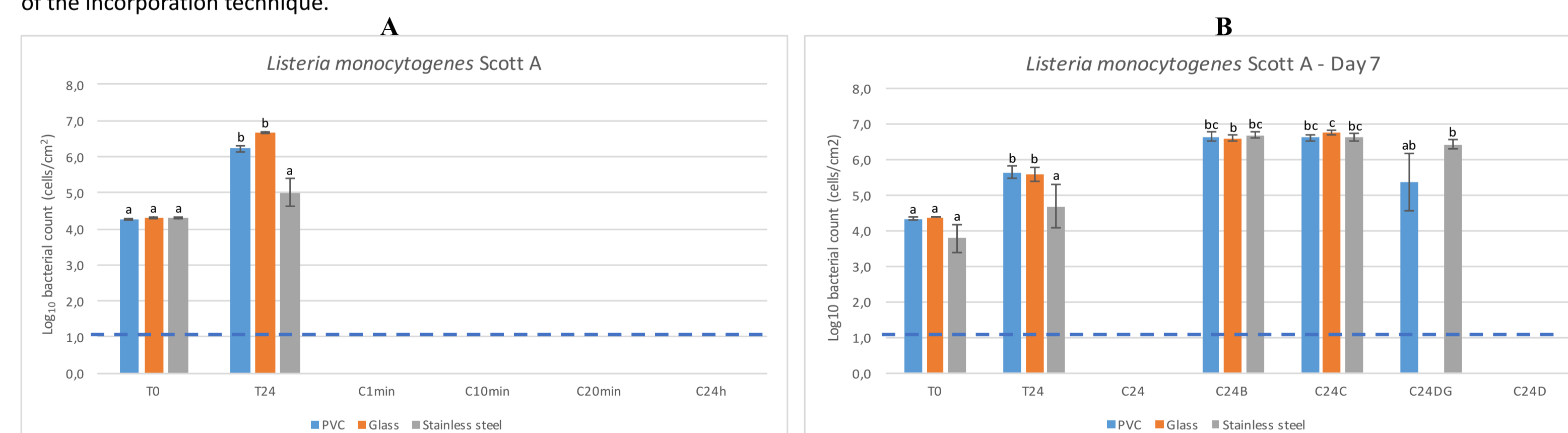


Figure 3. A) Contact killing time for *Listeria monocytogenes* Scott A. Recovery of bacteria immediately after inoculation (T0); after 24h incubation (T24) on untreated surfaces; Recovery of bacteria immediately after inoculation (C1min); after 10 minutes (C10min), 20 minutes (C20min) and 24h incubation (C24h) on each treated surface. **B)** Antimicrobial activity of the compound on day 7 for *Listeria monocytogenes* Scott A. Recovery of bacteria immediately after inoculation (T0); after 24h incubation (T24) on untreated surface; after 24h incubation (C24B) on treated surface cleaned with bleach; 24h incubation (C24C) on treated surface cleaned with damp cloth; 24h incubation (C24DG) on treated surface cleaned with commercial degreaser and 24h incubation (C24D) on treated surface cleaned with commercial disinfectant. The results are means based on data from three replicates and standard deviations are indicated by error bars. Equivalent lower case letters mean no significant differences between each condition ($p>0.05$). The dotted line means that the isolate was reduced to values below the detection limit of the incorporation technique.

Growth inhibition for all three tested pathogens was observed after a 1-minute contact with the treated surfaces for all surfaces tested (PVC, glass and stainless steel) (Figures 1A, 2A and 3A). Short contact killing time may be due to the fact that bacterial adhesion is inhibited being the cell lysed before attaching to the surface.

Antimicrobial activity (R) was assessed and compared to the control (T24). Durability of the antimicrobial activity on the treated surfaces was shown to be less than 7 days after surface cleaning since no growth was observed i) for all treated surfaces cleaned with commercial disinfectant and ii) for glass surfaces cleaned with degreaser for *E. coli* and *L. monocytogenes*.

Results and discussion

Antimicrobial product cytotoxicity – Trace concentrations on Caco-2 cells

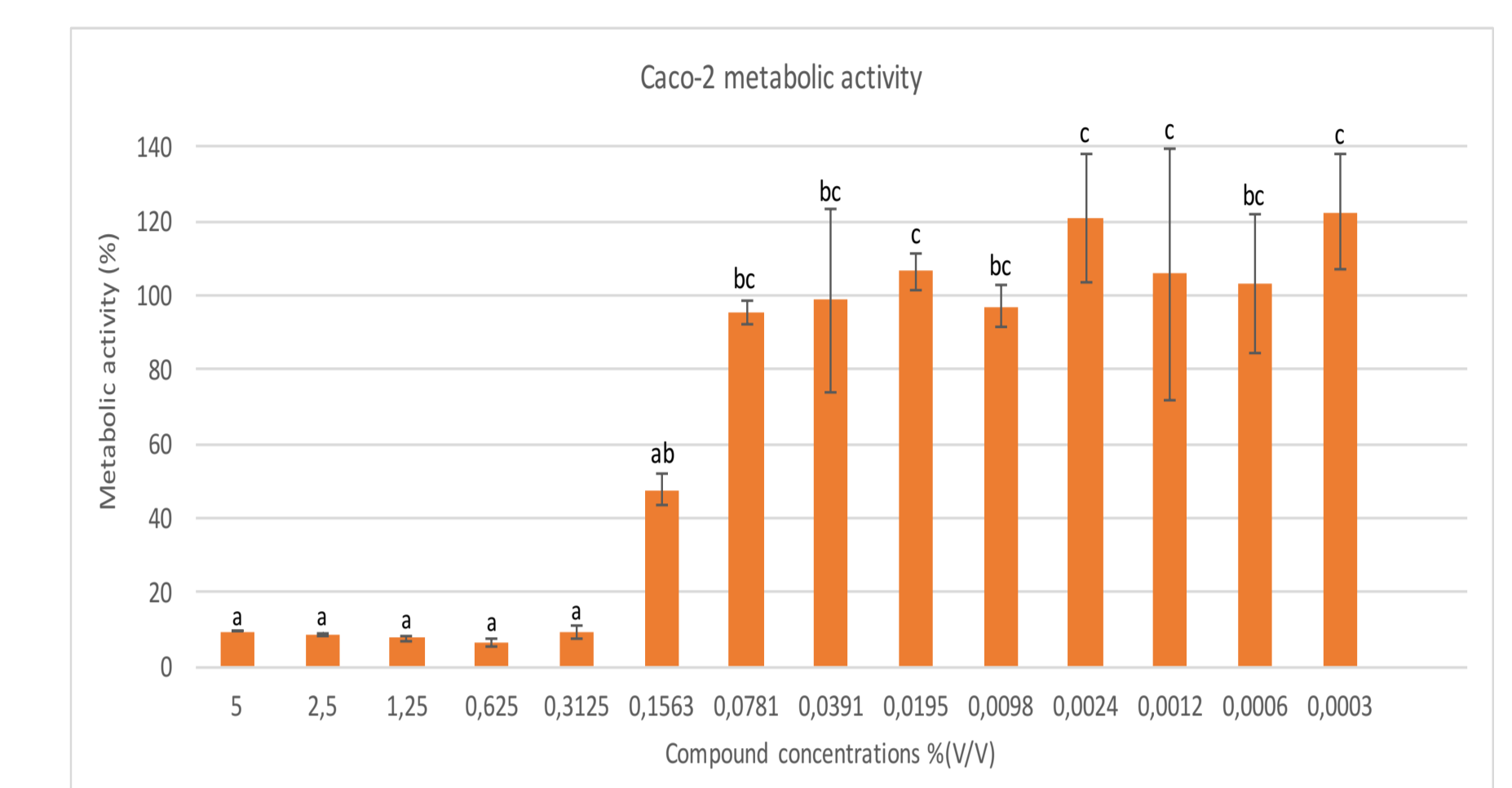


Figure 4. Metabolic activity of Caco-2 cells exposed to trace concentrations of the coating.

Trace concentrations of the antimicrobial product were tested to assess if antimicrobial migration to food products through food contact surfaces would be detrimental if ingested.

No vestigial concentrations of antimicrobial coating ($\leq 0.1563\%$ v/v) were cytotoxic to human colorectal adenocarcinoma cells.

Conclusion

After a 1-minute contact time bacterial growth was inhibited in all treated surfaces. Antimicrobial activity of the product was proven. Its durability was less than reported by the manufacturer, although for surfaces cleaned with the commercial disinfectant no growth was observed for any of the pathogens tested.

Regarding the product cytotoxicity, trace concentrations lower than 0.1563% v/v were not considered cytotoxic towards human colorectal adenocarcinoma cells (Caco-2).

Although promising, the preliminary results obtained in this study need to be complemented, extending the study to more pathogens and surfaces. Also, the efficacy of this antimicrobial coating when the pathogens are incorporated within a food matrix as well as their possible acquired antimicrobial resistance should be the focus of further studies.

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