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Bacterial biofilms control in the food industry using chemical complexes

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The biofilms formation favors the horizontal antibiotic resistance genes transfer and is also one of the main causes of food spoilage, outbreaks of foodborne infections and biofouling of equipment in the food industry, causing serious damage to the sector. Controlling the biofilms presence associated with pathogens of unique health importance requires an understanding of their ultrastructure and how different sanitizing agents work. Our approach included evaluating the three-dimensional organization and composition of biofilm matrices of four multidrug-resistant bacterial species (*Campylobacter jejuni* - CJ, *Escherichia coli* pathogenic for poultry - APEC, *Salmonella* Choleraesuis - SC, *Salmonella* Typhimurium - ST) isolated from the food industry and three reference strains (*Escherichia coli* ATCC 25922 - EC, *Salmonella* Enteritidis ATCC 13076 - SE, *Staphylococcus aureus* ATCC 25923 - SA), as well as determining the effect of innovative chemical products (P1- vegetable d-limonene solvent + quaternary, P2- quaternary + phosphoric acid, P3 -blend of enzymes - protease and amylase, P4- hypochlorous acid) on the biofilm formation inhibition and the extracellular matrix degradation. Scanning Electron Microscopy determined the biofilms ultrastructure and Fluorescence Microscopy provided the qualitative parameter for characterizing the protein and carbohydrate fractions of the extracellular matrix. The enzymatic method was used to determine the fractions of carbohydrates, extracellular deoxyribonucleic acid (DNAe) and matrix proteins. The fraction with the highest content in the strains biomass alternated between protein and carbohydrates. The highest percentage of carbohydrates was identified in SE (61.5%). The biomass with the highest DNAe content was found in SE (57.3 %), while the highest protein content was observed in SC (77.1 %). Regarding the efficiency of the chemicals tested, we observed that the products were more effective at inhibiting the formation than degrading the mature biofilm. P4 was able to inhibit all the strains, with the highest inhibition percentages for biofilm formation. P1 and P3 were also able to significantly inhibit all strains with varying percentages of inhibition, reaching 79.8% and 71% in EC, respectively. P2 was the product that showed the lowest inhibition capacity, even showing an inverse action in SE and ST, favoring the formation of biofilms in percentages of 56 % and 132 %, respectively. The ability to degrade mature biofilm was strain-dependent, with values varying according to the microorganism evaluated. P1 acted significantly on the matrix, with percentages ranging from 3.9 % in APEC to 50.5 % in SA, with non-significant degradation in EC, SC and ST. P2 degraded the bacteria biofilms evaluated in varying percentages, but had no effect on EC and ST and also increased the biomass of the biofilms formed by SC and SE by 10 % and 67 %, respectively. P3 reduced the biomass of SA by 81 %, but increased it by 29 % in CJ. P4 was efficient in degradation except for CJ, APEC and SA where the reductions

were not significant. The comparison of the results of biofilm formation and degradation inhibition leads to the conclusion that there is protection against sanitizers mediated by the matrix already formed, and thus the use of preventive rather than corrective procedures to mitigate their various harmful effects on public health and the economy is more effective.

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