Scientific report

on the implementation of the project

"Recovery of Chitinous Material from Recyclable Waste by Using it in Several Potential Biological Applications (ReWaChi)

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Establishing an optimal chitosan based formula for inhibiting cell growth (human cell line cultures) and microorganisms for extended applications

Abstract

The studies performed this year led to the following conclusions:

- The study included in vitro *testing* of the impact of chitosan solutions obtained by controlling extraction and purification parameters, thus 15 experimental variants were analyzed and biologically evaluated. Based on the first results obtained in *in vitro* testing (cultures, human cells) and in *in vivo* testing (two biotester organisms, used in toxicity, ecotoxicity, aquaculture applications), new evaluation protocols were created using chitosan mixtures as well as different formulations of chitosan (CH):chitin (CHT):oligomers. Formulations by grafting cerium (Ce) ions onto chitosan were also included in the testing.
- Tests to assess the impact induced by these chitosan formulations consisted of analyzing the ability to survive and cell proliferation of normal and tumor cell lines using the colony formation assay (Clonogenic assay), as well as determining the expression levels of chitinase-like YKL40 protein, in order to establish the therapeutic efficacy of these nanocompounds. Epithelial cells from the mammary gland, MCF-12A (ATCC CRL-3598) and tumor epithelial cells from a uterine cervix adenocarcinoma, HeLa (ATCC CRM-CCL-2), were analyzed. The SK-MEL-28 melanoma cell line (ATCC HTB-72) was used to evaluate the expression of chitinase-like YKL-40 protein.
- Studies to identify how the biological activity of microorganisms is influenced by the presence of chitosan with different molar mass and in correlation with the variation of concentrations of tested samples started from the information that the mechanisms manifested differently on biological systems (bacteria, fungi) depend on the structure of exposed biological systems and the polymer target that can be at cellular or molecular level (interaction between chitosan and components membrane, the mode of penetration into cellular structures). The effects induced by the presence of chitosan molecules with different molar masses and degrees of deacetylation on three strains of bacteria with different structural properties as well as with varied pathogenic action and virulence, strains of interest in the field of public health, were analyzed. Thus, the effects on a gram-positive species *Staphylococcus aureus* (ATCC 23235) were analyzed and from the gram-negative category were evaluated *Pseudomonas aeruginosa (ATCC* 27353) and *Escherichia coli (ATCC 25922*). Two species of the genus Candida were used to test the antifungal action*: Candida albicans ATCC* 10231 and *Candida parapsilosis* ATCC 22019. Following the experiments performed, the following aspects were found: chitosan induces inhibition of the bacterium *S. aureus*, depending on the molar mass, thus, in samples with molar mass between 170 and 413 kDa, antibacterial effects start at the concentration of 50 μg / mL. Chitosan with molar mass between 475 and 992 kDa induces bacterial inhibition at concentrations greater than 200 μg/mL. These observations can be linked to explanations in the literature that smaller particles can enter cells faster. No bacterial inhibition phenomena have been identified in chitosan molecules with low molar mass of 26.30 kDa (C4) and 155 kDa (C16). The chitosan samples tested had effects close to bactericidal concentrations of comparatively analyzed antibiotics, which offers promising prospects for future applications.
- Another aspect analyzed was the adhesion of these microorganisms to chitosan-based films. Thus, membranes obtained from chitosan of 0.5%, 1%, 2% concentrations, in different solvents (acetic acid and lactic acid) and under different temperatures, were exposed to bacterial

cultures. It was evaluated how these microorganisms retain adhesion to membrane surfaces or not, which is important for biomedical applications of chitosan, but also in other applications such as obtaining packaging or filters for water. In our study, membranes obtained at low temperatures had a total inhibitory effect at variable concentrations of chitosan on all bacteria. As a result, the effect of temperature in the process of configuring membranes is as important as the concentration of the polymer.