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CO-CULTIVATION OF *HAEMATOCOCCUS PLUVIALIS* AND *CHLORELLA* SP. AS A NOVEL STRATEGY FOR MICROALGAL-BASED BIOTECHNOLOGY

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Microalgae have been identified as potential sources of valuable products with many commercial applications including food supplements, feed additives and biofuel feedstocks. They are innovative production platforms since, in order to adapt to growth conditions, they synthesize various metabolites. However, the synthesis of these biomolecules requires an adequate selection of microalgal species, a deep knowledge of their biology and physiology, as well as rigorous evaluation of cultivation strategies. Monocultures have been the preferred production route in the bio-industry. Nevertheless, from a biotechnological perspective, it is necessary to develop successful cultivation technologies to increase their productivity, in terms of biomass and availability of biomolecules. In this way, there is increasing interest in the use of co-cultures to deal with contamination issues, and simultaneously increase productivity and product diversity. Thus, in this work our purpose was to analyze co-cultivations of two different microalgal strains in terms of biomass production and product availability. For this end, *Haematococcus pluvialis* and *Chlorella* sp., two important carotenoid producers, were selected for co-cultivations in an appropriate culture medium at 22°C for 10 days. Then, cell number, dry weight, chlorophyll and carotenoid quantification and autofluorescence, Red Nile (RN) fluorescence, and triacylglyceride (TAG) and sterol contents were analyzed. The results revealed that co-cultivation based on 50% *H. pluvialis* and 50% *Chlorella* sp. prevented population domination of one strain over the other. In addition, this co-cultivation condition showed the highest values in terms of cell density and dry weight. Flow cytometry analyses also shown the maximum RN fluorescence and carotenoid autofluorescence within this experimental condition. In addition, in co-cultures based on 50% *H. pluvialis* and 50% *Chlorella* sp., carotenoid autofluorescence was accompanied by the greatest increase in the antioxidant capacity and in the amount of total carotenoids. Moreover, thin layer chromatography coupled to spectrophotometric quantification also showed highest TAG and sterol contents. The results suggest that the co-cultivation system based on 50% *H. pluvialis* and 50% *Chlorella* sp. may be a successful strategy to enhance biomass yield and the obtention of value-added products, supporting the development of a microalgal-based biotechnological process.

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DESIGN, CONSTRUCTION AND PURIFICATION OF A CHIMERIC S-LAYER-*TRYPANOSOMA CRUZI* PROTEIN FOR IMMUNOPROPHILACTIC APPLICATIONS AGAINST CHAGAS DISEASE

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Chagas disease (CD) is an endemic malady in Argentina and there are no vaccines for human application. Though heterologous expression of specific antigens in generally recognized as safe bacteria (GRAS) represents a tempting alternative for vaccine formulations, the engineering of Gram positive strains represents a real challenge. In this work, we present a similar approach that combines the immunogenicity of a specific antigen of *Trypanosoma cruzi* with the beneficial adjuvant properties of a probiotic bacterium. In order to obtain a system for antigen- self-assembly that enables spontaneous adhesion on multiple surfaces, we developed a genetic construction. For that, we engineered a structure-based chimeric antigen between the SpyTag peptide, a bond-forming subunit of *Streptococcus pyogenes* followed by the N-terminus fragment of Tc52 (N-Tc52), an immunogenic protein of *T. cruzi*, and SlpA, a surface layer protein of *Lactobacillus acidophilus*. The final transcriptional fusion was carried out by successive asymmetric PCRs. In the first step, the sequence encoding to N-Tc52 was amplified by PCR from *T. cruzi* CL Brener strain using specific primers to incorporate cloning sites, the sequence encoding to SpyTag and a fragment of SlpA gene. In the second step, the gene encoding to SlpA was amplified by PCR from *L. acidophillus* ATCC 4356 using specific primers to incorporate cloning sites and a fragment of N-Tc52. Finally, we fused the obtained genes by using different combinations and concentrations of primers in an asymmetric PCR. Once obtained, the final fragment was cloned in pRSET-A and inserted into *Escherichia coli DH5α.* The recombinant plasmid containing the hybrid gene *6His-SpyTag-NTc52-SlpA* was purified and inserted into *E. coli* BL21 [DE3]. Expression of the 6His-SpyTag-NTc52-SlpA protein was carried out by the addition of IPTG 1 mM at 28 ºC. After 4 h of induction, cells were collected by centrifugation in phosphate buffered saline and lysed by repetitive cycles of sonication, freezing and thawing. Subsequently, the lysate was centrifuged and the pellet, containing the protein in inclusion bodies, was resuspended in Urea 8M. The protein was purified through a Ni-NTA agarose cartridge.

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EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF BACTERIOCINS PRODUCED BY REGIONAL *YERSINIA* STRAINS

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