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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.

BT-P03-95

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 °C. 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.

BT-P04-108

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

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Bacteriocins are extracellular peptides of ribosomal origin and encoded at the plasmid level. Pathogenic *Yersinia enterocolitica* strains belonging to biotypes (B) 1 to 5 are cause of gastrointestinal symptoms and immunological sequelae in humans by consumption of contaminated foods. *Y. frederiksenii*, *Y. intermedia* and strains of *Y. enterocolitica* B1A do not carry the virulence markers that characterize pathogenic *Y. enterocolitica* strains; however, they may produce bacteriocins that inhibit the growth or destroy these pathogenic strains. To contribute to food safety and the human health, the use of bacteria as biocontrol agents in food has been proposed since they offer safe advantages to the consumer. The aim of this study was to evaluate the antimicrobial activity of bacteriocins produced by *Yersinia* strains on pathogenic *Y. enterocolitica* strains isolated from various foods in our region by the plate titration method. Two Y. *intermedia* B1 (named 79 and 26), one Y. *intermedia* B6 (10) and three *Y. enterocolitica* B1A (66, 89, 90) were tested as bacteriocin-producing strains (BPS), and three *Y. enterocolitica* strains belonging to B2, B3 and B4 were used as indicator strains (IS) of the antimicrobial effect. The spot technique was performed on a double layer agar. BPS and IS were cultured in Luria Bertani broth (LB) with shaking at 25°C for 18 h, and inocula were adjusted to a concentration corresponding to an $OD_{\lambda 610}$ 0.2. From BPS, two-fold dilutions were made in LB, and 10 µl of each one was placed on Petri plates with semisolid agar previously inoculated with IS. Plates were incubated at 25°C and at 10°C for 18 h. The reciprocal of the highest dilution of BPS that produced total inhibition of IS was considered as the titer and expressed in arbitrary units per ml (AU ml^{-1}). Results represent the average of three different experiments. At 25°C, the highest inhibition titers were observed for *Y. enterocolitica* B1A (90) on *Y. enterocolitica* B2 and B4, with values of 12,800 $\pm$ 0 AU ml^{-1} and 10,667 $\pm$ 3,695 AU ml^{-1} ($p \geq 0.05$), respectively. At 10 ° C, the highest inhibition titers were shown by *Y. intermedia* B1 (79) on *Y. enterocolitica* B2 and B4, with values of 9,262 $\pm$ 3,695 AU ml^{-1} and 6,400 $\pm$ 0 AU ml^{-1} ($p \geq 0.05$), respectively. All BPS showed lower titers on *Y. enterocolitica* B3 than on *Y. enterocolitica* B2 and B4 ($p \leq 0.05$) at both temperatures. When comparing the two temperatures, most of titles produced by BPS were higher at 25°C than at 10°C ($p \leq 0.05$). Results obtained in this study demonstrate the capacity of regional *Y. intermedia* and *Y. enterocolitica* B1A strains to produce significant amounts of bacteriocins with inhibitory effect on pathogenic *Y. enterocolitica* strains, and highlight the great potential of these substances as antagonists of pathogenic or spoilage bacteria in food, even at refrigeration temperatures.

BT-P05-112

A PERMEABLE TETANUS TOXIN IS CAPABLE OF TRANSLOCATING INTO THE OOCYTES AND ALTER THE CORTICAL GRANULES EXOCYTOSIS

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The cortical reaction in oocytes is a fundamental process that occurs during gamete fusion at fertilization. It entails the exocytosis of cortical granules and is involved in blocking polyspermy. There are multiple proteins that intervene in the fusion of cortical granules and plasma membrane, among which proteins of the VAMP family can be mentioned. It has been proven in our laboratory that by microinjecting tetanus toxin (TxTe), VAMP proteins are cleaved, and cortical granules exocytosis (CGE) can be avoided. On the other hand, cell penetration components are molecules that can cross plasma membranes due to characteristics of their chemical nature. They have received attention as biotechnological tools because of their ability to transport useful substances to cells. However, the ability of these components to enter the cytoplasm of oocytes is unknown. To determine if permeable components can be used as biotechnological tools to deliver molecules into the oocytes, the aim of this work was 1) to study if a permeable tetanus toxin (p-TxTe) was able to cross the zona pellucida and the membrane of mature oocytes (MII), and 2) to analyze if this toxin might inhibit the cortical granule exocytosis (CGE). Permeable TxTe bound to a cell-penetrating peptide with multiple arginine residues was purified from transformed bacteria *Escherichia coli*. MII oocytes were collected from hormonally stimulated female CF-1 mice, and incubated in p-TxTe at different times, at 37 ° C. Then, a group of oocytes was fixed and treated with primary and secondary antibodies to perform an indirect immunofluorescence assay (IFI). From the analysis of cells by confocal microscopy, it was determined that p-TxTe was able to translocate into the oocytes, through the zona pellucida and the plasma membrane. In turn, to analyze if CGE had been affected by incubation in TxTe, oocytes were parthenogenetically activated by strontium chloride ($SrCl_2$). Compared with control oocytes, oocytes treated with p-TxTe were not able to respond to the activator. These results show that p-TxTe inhibited cortical granules exocytosis and that permeable components can be used as biotechnological tools to deliver useful molecules into the oocytes.

BT-P06-118

HETEROLOGOUS PROTEINS DISPLAY ON LACTIC ACID BACTERIA BY USING THE *Lacticaseibacillus paracasei* PHAGE PL-1 ENDOLYSIN

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