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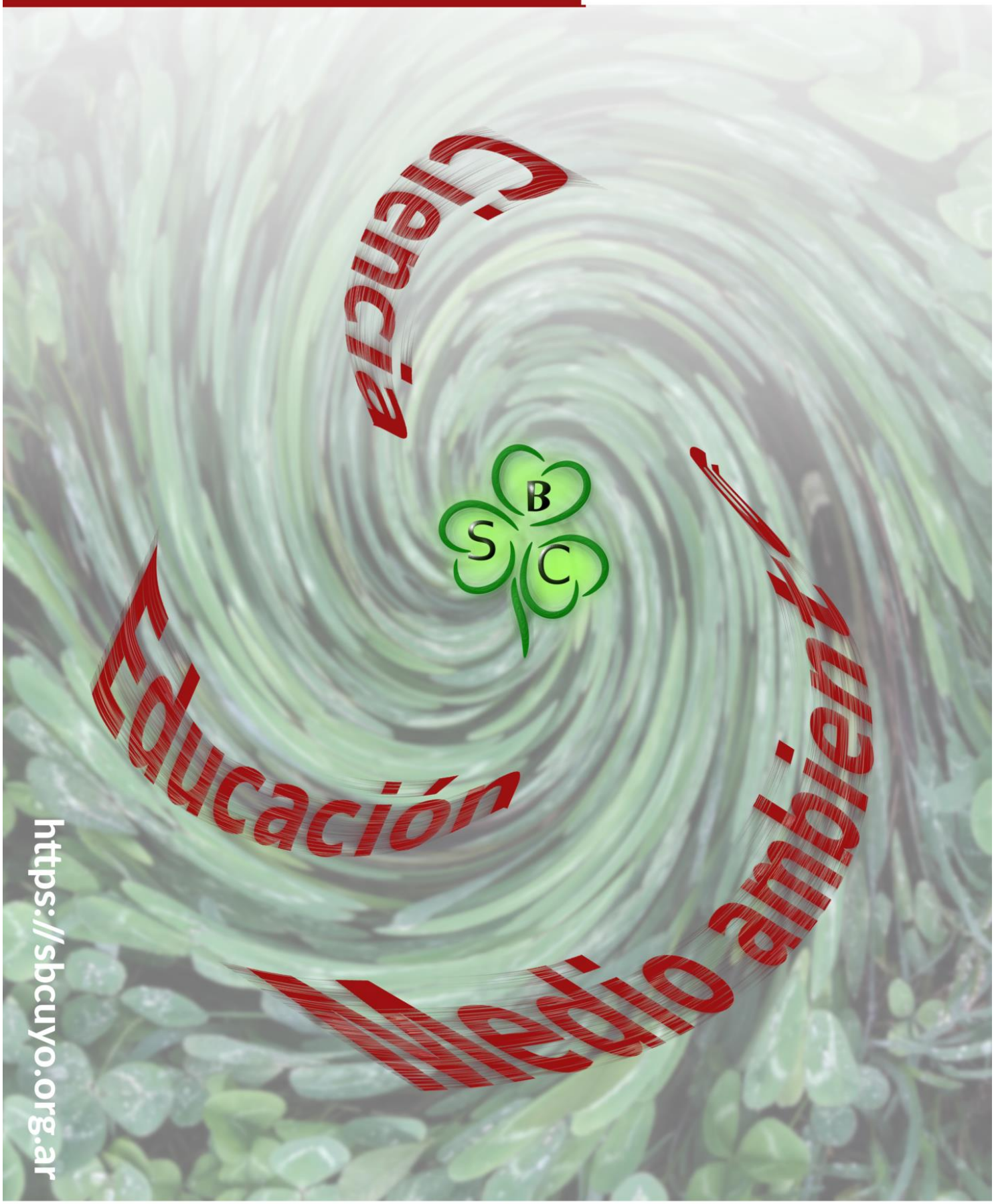

markers neither the activity of the MMP-2 enzyme and its zymogen. In relation to the inflammation factors, the expression of IL-1β and TNFRp55 did not modify, while the expression of TNF-α and TNFRp75 decreased significantly in endometriotic lesions in comparison with the control group ($p<0.05$). In addition to promoting the inflammatory process, TNF-α cytokine contributes to the maintenance of functional nerves and associated pain in EDT. Therefore, the results obtained would indicate that progesterone, although it seems not to alter apoptosis and invasion, can have a beneficial effect on this pathology by modulating the expression of the TNF-α-TNFRp75 signaling system.

46. EFFECTS OF PRENATAL STRESS AND POSTNATAL ACUTE FORCED SWIMMING ON SOME BIOCHEMICAL PARAMETERS OF LIVER AND SKELETAL MUSCLE

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The aim of this study was to analyze the physiological response of adult rats to prenatal stress and postnatal acute forced swimming on skeletal muscle and hepatic parameters. Males of three months of age were used, controls individuals (PC) and some prenatal stressed for immobilization (IMO) during pregnancy (PS). Half of the PS and PC animals were summited to the postnatal forced swimming stress section for 30 min. Corticosterone (COR), glycemia and malondialdehyde (MDA) levels were studied. Our results showed that in basal conditions COR and glycemia levels are increased in PS animals. Also, after an acute exposition of forced swimming, COR significantly increases in the PS group in the same way as in PC rats but with minor effects. The hepatic MDA levels, in control condition, showed a rise in PS rats compared to PC animals, being the difference maintained after forced swimming stimuli. In skeletal muscle, MDA values of PS animals were increased in basal conditions, but this effect was observed in all groups after forced swimming treatment. In conclusion, IMO prenatal stress causes a change in the regulation of the HHA axis, reflected in high basal levels of plasmatic COR and hyperglycemia. Moreover, it produces a hypersensibility to acute stressors during postnatal adult life. On the other hand, PS and forced swimming stress generate high levels of oxidative stress in the liver and skeletal muscle.

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47. UPGRADING GRAPE AND OLIVE POMACES BY PECTINOLYTIC ENZYMES PRODUCTION: OPTIMIZATION OF CULTURE CONDITIONS

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In the wine and olive Cuyo region industries, large masses of grape pomace (GP), grape stalks, alperujo and olive pomace (OP) are produced. These wastes have polluting characteristics such as low pH and high content of phytotoxic and antibacterial phenolic substances, which hinder their biological degradation. According to current legislation, they must be treated for safe final disposal. Solid State Fermentation (SSF) is biotechnological option to revalue them, by generation of value-added products (for example, enzymes). SSF performance depends on many variables, and then it is important to state the relevance, for each of them, when a certain bioproduct is the objective. In a previous work, for the production of a Pectinolytic Enzyme Complex (PEC) by *A. niger* SSF, relevant variables were found. The objective of this work was to optimize the value of these relevant variables, for maximum PEC production. The Box Behnken Design (BBD) was used for the variables: glucose (G), micronutrient solution (MS) and initial humidity (H); as response, enzymatic activities (Exo-polygacturonase (Exo-PG), Endo-polygacturonase (Endo-PG) and Exo-polymethylgacturonase (Exo-PMG)) were analyzed. The BBD requires setting three levels: low, medium and high. The solid substrate was placed in flasks (50% GP and 50% OP) corresponding to a BBD test. Fifteen SSF was carried out for 3.5 days at 27°C with an inoculum of $1x10^7$ spores/g_{dry}. Enzymatic extracts from SSF were prepared in Na-acetate buffer (0.1 M, pH 5). The Exo-PG and Exo-PMG activities were determined at 45°C by spectrophotometric methods and Endo-PG, by viscosity monitoring via Ostwald viscometer. Responses obtained (enzyme activities) were used for quadratic polynomial model, ANOVA and response-surfaces construction, using the Design-Expert 11-Stat-Ease^{MR} program. The optimal values of the variables for maximum PEC (simultaneous Exo-PG, Endo-PG and Exo-PMG production) were: 1.5% G, 5% MS and 66% H. The maximum enzyme activity values predicted by the model were: Exo-PG: 0.87 U/g; Endo-PG: 0.82 U/g and Exo-PMG: 0.77 U/g. When validating these results with SSF trials, it was observed that the maximum activity was: Endo-PG 26% more than predicted, Exo-PMG 33% less, both at 3.5 days, while for Exo-PG obtained 34% more than predicted after 3 days.

48. CLONING, OVEREXPRESSION AND PURIFICATION OF MUR ENZYMES OF *Brucella abortus* FOR STRUCTURAL RESOLUTION BY X-RAY CRYSTALLOGRAPHY

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Peptidoglycan's biosynthesis is one of the most studied mechanisms in microbiology. It consists of two stages, a cytoplasmic phase dependent on the Mur protein family and a periplasmic phase controlled by the Penicillin Binding Proteins (PBPs) responsible for the formation of the bacterial cell wall. The PBPs have been the target for the search and design of antibacterials since the discovery of penicillin. However, with the increasing acquisition of resistance mechanisms by bacteria, introduction of novel classes of antibiotics is necessary. On this line, drugs have been clinically incorporated which alter significantly other elements of the biosynthetic machinery as the case of fosfomycin, an inhibitory antibiotic of MurA. This protein, along with the rest of the Mur family, is responsible for synthetizing the UDP-N-acetyl-D-muramate pentapeptide (or UDP-MurNAc-pentapéptide), the precursor of peptidoglycan. As Mur enzymes are highly conserved in bacteria and there has not been found a crossed reaction with the eukaryotic cells, it is expected that inhibitors of Mur proteins may be potentially broad spectrum bactericidal compounds. On this work we focus on Mur proteins of *Brucella abortus*, the etiological agent of Mediterranean Fever, a world-wide distribution zoonosis. We have amplified the MurF gene from *B. abortus* strain with PCR, cloned inside an expression vector pET28a, expressed and purified to homogeneity by QIAGEN© Ni-NTA affinity resin. Now, we are running crystallization assays in order to solve its 3D structure by X-ray diffraction. This would help to the rational design of new inhibitors against brucellosis.

49. LIGNINOLYTIC ACTIVITIES PRODUCTION IN SOLID STATE FERMENTATION USING OLIVE POMACE. SCREENING OF VARIABLES FOR ITS OPTIMIZATION

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Alperujo (AL) is a by-product after two-phase extraction process of olive oil. AL is semi-solid, and its composition by weight is: water 83-96%, organic matter 3.5-15% and mineral substances 0.5-2%. The organic fraction includes sugars, proteins, organic acids, lipids, pectin and phenolic compounds (PC). Due to its PC content, AL is often considered as a toxic by-product. In addition, because of legal requirements, it is necessary to carry out a detoxifying treatment prior its final disposal. Solid State Fermentation (SSF) is a treatment that provides simultaneously detoxification and valorization for AL. Through some preliminary SSF tests, using culture medium made of olive pomace (obtained from AL) and filamentous fungi isolated from AL, microorganism growth, PC consumption and ligninolytic enzyme production were verified. In the present work, the aim was to study the effects of some variables in Lignine-Peroxidase (LiP) and Laccasa (Lac) production in solid-state fermentation of olive pomace, obtained from AL. For this, a statistical test was made using Plackett-Burman Design (PBD) for four variables, at two levels (low (-) and high (+)). Variables were X_1: Aqueous Extract from AL (0 and 1 % p/p), X_2: Veratryl Alcohol (0 and 0.02 % p/p), X_3: Initial water content (55 and 65 %), and, X_4: Mixing (No and Yes). responses were LiP and Lac activities. The eight SSF essays according PBD, were performed in a bench scale fixed bed reactor. This reactor has an internal tray of 28 cm diameter and 10 cm high, which was eight-portion fractioned, for each one of the essay. Reactor was set in a room during 15 days, at controlled 27 °C and humidified forced aeration was provided. Culture medium was prepared with olive pomace (70 % p/p) and grape stalks (30 % p/p); inoculum was 10^7conidia/g_{dry}. Enzymatic activities were quantified by spectrophotometric methods, using Veratryl Alcohol and ABTS as substrates for LiP and Lac respectively. Samples for response determination were taken at day 3, 5, 7, 10 and 15. Results shown, at 7 days (where better enzyme activities were found): For Lac and LiP production, the increase of X_3 variable show a positive effect, while X_1, X_2 and X_4 show a negative effect when they went from the low level to the high one. In case of LiP production, X_3 was significant at 80 % confidence level, and for Lac, 95 %. The rest of variables, shown significance with different confidence levels ranging 90% to 95% for LiP, and 80% to 99% for Lac. Main conclusion is that only Initial Humidity Content (X_3) positively affects both enzyme activities. Future essay would be aim to optimized variables whose effects were positive and to search for new inducers for both enzymes

50. ANTIMICROBIAL ACTIVITY AGAINST *Escherichia coli* OF NANOPARTICLES OBTAINED BY DIFFERENT SYNTHESIS METHODS

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Escherichia coli, is a pathogenic bacterium that causes serious infections, whose therapeutic treatment is threatened by the emergence of multiple resistance to conventional antibiotics. In recent years, metal nanoparticles (NP) have been studied for their antimicrobial capacity and its possible applications as an alternative to antibiotics against different pathogens. The NPs also vary in synthesis techniques; either by chemical, physical and biological based methods. The objective of this work was to study the possible antimicrobial capacity of nanoparticles obtained by methods chemical and biological against *E. coli*. The NPs obtained by a method chemical called citrate- gel (Quim-NP CuNi B1-300; Quim-NP CuNi B1-500; Quim-NP CuNi B3-300) and NPs obtained by a method biological, silver nanoparticles (AgNPs), synthesized by biological mediators such as, *Cryptococcus laurentii* (AgNPs-C.l) and *Rhodotorula glutinis* (AgNPs-R.g). The antimicrobial capacity of the aforementioned NPs was evaluated in vitro by means of the agar diffusion method; 200 µL of an *E. coli* (ATCC 8739) suspension, at a concentration of $3x10^8$ CFU mL^{-1}, were seeded in 10 cm Petri dishes with selective medium and incubated for 1 h at 37°C±1. Subsequently, wells of 3 mm were made aseptically and were filled with 25 µL of the suspensions of AgNPs-R.g, AgNPs-C.l, CuNi B1-300; CuNi B1-500, CuNi B3-300 and the combination thereof. Distilled water was used with negative control. Then they were incubated for 48 hours at 37°C±1. After incubation, the zones of