BIOCELL 2013, 37(2): A35 - A79 ISSN 0327 - 9545 PRINTED IN ARGENTINA



TUCUMAN BIOLOGY ASSOCIATION

(Asociación de Biología de Tucumán)

Abstracts from the

XXIX ANNUAL SCIENTIFIC MEETING

October 17-19, 2012 Horco Molle, Tucumán, Argentina

The abstracts have been revised and evaluated by the Scientific Committee of the Tucumán Biology Association

25.

PHYTOCHEMICAL CHARACTERIZATION AND POTENTIAL USE IN VETERINARY MEDICINE OF PROPOLIS FROM ARID REGIONS OF NORTHWESTERN ARGENTINA

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Introduction: In previous reports we demonstrated the antibacterial activity of propolis hydroalcoholic extracts from arid and semi-arid regions of northwestern Argentina against human pathogens. The objective of this study was to compare two extraction methods of propolis from arid regions and evaluate their antibacterial activity against bacteria isolated from canine otitis. Materials and methods: Propolis extracts were prepared by successive extractions and maceration using ethanol 80°. Extracts were characterized by TLC and HPLC-DAD, and total phenolics compounds, flavonoids and non flavonoids were determined by spectrophotometric methods. Minimal inhibitory concentration (MIC) values were determined by the agar macrodilution method against 11 strains isolated from canine otitis (Staphylococcus and Proteus). Results: Four compounds (two chalcones, one flavone and one flavanone) were identified. Propolis tincture showed MIC values of 33 µg/ml and 267 µg/ml for the Grampositive and Gram-negative bacteria, respectively. Conclusion: the propolis from arid regions of northwestern Argentina may be used in veterinary medicine.

26.

SEQUENTIAL AND SIMULTANEOUS INOCULATION OF *Oenococcus oeni* WITH MIXED CULTURES OF WINE YEASTS: METABOLISM OF SUGARS AND MALATE

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Alcoholic and malolactic fermentations by Saccharomyces and O. oeni contribute to the organoleptic profile of wines. This work also includes an apiculate yeast to determine the metabolic resources of S. cerevisiae mc., K. apiculata mF and O. oeni X₂L to grow in grape juice medium. Yeast mixed cultures were performed as follows: 1-mF 10⁶-mc, 10⁶ CFU/mL, 2-mF 10⁴-mc, 10⁶ CFU/mL. Media were fermented, filtered and inoculated with 106 CFU/mL-X₂L (sequential culture-SC). Simultaneous cultures included mF, mc, and X₂L strains (106 CFU/mL each). All the cultures were incubated at 30°C in microaerophilia. At different time intervals, samples were taken for analytical determinations. Yeasts consumed 91 (1) and 96% (2) of sugars at 48h, yielding 159 mM ethanol, 16 mM acetate, 4.8 mM lactate and 3.6 mM glycerol at 144h; carbon recovery (CR) was 91%. X,L strain grew in SC. Malate was consumed and 4.9 mM lactate, 0.34 mM acetate, 3.5 mM ethanol and 1 mM glycerol were detected (CR=96%).

In simultaneous cultures the strains consumed 96% of sugars and 65.9% of malate at 72 h and produced 157 mM ethanol, 19.7 mM acetate, 7.7 mM lactate and 2.54 mM glycerol (CR=98%). The results allow us to propose the inoculation conditions that can drive both fermentations without modifying the wine quality.

27.

OPTIMIZATION OF BIOMASS AND BACTERIOCIN PRODUCTION BY *Lactococcus lactis* CRL 1584, A POTENTIAL PROBIOTIC FOR RANICULTURE

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L. lactis CRL 1584, isolated from a bullfrog hatchery, inhibits etiological agents of red-leg syndrome and *Listeria monocytogenes* by organic acids, H_2O_2 and bacteriocin. Thus, *L. lactis* is a beneficial bacterium for raniculture that should be administered to the host at viable high numbers to obtain a beneficial effect. Then, a response surface model was applied to optimize both biomass and bacteriocin production in LAPTg medium at 36°C. Biomass was determined by OD_{540nm} and bacteriocin activity by the plate diffusion method, expressed as Log AU/mL.

In the central point of the design, biomass value was 1.14 and bacteriocin 2.56. By using the response surface model for biomass, peptone and tryptone exerted a positive linear effect and a negative interaction between the two. Yeast extract (EL) exerted a linear positive effect and glucose a quadratic negative effect, the maximum being 6.25 g/L glucose. With respect to bacteriocin production, peptone and EL showed a linear positive effect and a positive interaction with glucose. Optimal production of biomass (1.406) was obtained with (g/L): 18.74 peptone, 12.49 tryptone and EL and 6.25 glucose, while optimal bacteriocin production (3.39) was 18.74 peptone, 12.49 tryptone, EL and glucose.

This work provides the basis for biomass and bacteriocin production by *L. lactis* CRL 1584.

28.

CULTURE MEDIUM AND TECHNIQUES FOR THE DETECTION OF BIOFILM FORMATION BY *Enterococcus* faecalis. IN VITRO STUDY

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Enterococcus faecalis is the predominant species in root canals treated for persistent periapical lesion. The aims of this work were: A) evaluation of two culture media for biofilm development; B) biofilm detection by three fixation techniques. Materials and Methods: We evaluated three strains of Enterococcus faecalis isolated from the root canal. A) The media used were 1) Luria broth and 2) TS broth supplemented with 1% glucose and 10% human serum. B) Detection of biofilm was made by 1) staining with crystal violet at 10% and elution with alcohol (biofilm without fixation); 2) staining with crystal violet at 10% with heat-fixed biofilm and fixed with formaldehyde. The reading was performed using a microplate reader Versamax Microplate Reader (USA). Results: A) In Luria Bertani broth medium, no biofilm development was observed after 48 h of incubation. In TS broth culture medium supplemented with 1% glucose and 10% human serum, biofilm development was observed after 48 hours of incubation. B) Biofilm fixation using formaldehyde was significantly higher than the one performed with heat and without fixation (ANOVA p<0.0001). Conclusions: TS broth culture medium was appropriate for biofilm development of E. faecalis. Prior fixation with 10% formaldehyde was the most appropriate technique for biofilm detectiod.

Partly supported by CIUNT.