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06756

The extracts were: *T. guianensis*; *A. fraxinifolium*; *X. aromatica*; *G. viburnoides*; *V. poly*
A. humilis. Each pool of gel was loaded with 0.1g/mL of MMP-2. Gels were sectioned and placed into
solutions with the different extracts. To stimulate the proteolytic activity of gelatinases, a portion of the
gel activation buffer was incubated. Other portions were placed into the plant extracts under the same
conditions to evaluate the inhibitory effects of the tested products. Subsequently, the gels were stained
and soon after, bleached. The gels were photographed and the bands analyzed by densitometry system
for pixel analysis. **Results:** The proteolytic activity of gelatinases can be viewed by clear bands on dark
background of the gels. Thus, the observed bands size are inversely proportional to the inhibitory activity
by the tested extracts. It was found that extracts derived from plants selected a cted in different ways in the
gelatinases activities. The crude extract at the concentrations used in *T. guianensis*, *A. fraxinifolium*
and *X. aromatica* inhibited the activity of gelatinases in 85.7%, 87.1% and 80.6%-respectively. Extracts
of *G. viburnoides*, *V. polyanthus* had a lower inhibition of the gelatinases activities, 75% and 74%
respectively. Finally, *A. humilis* extract inhibited the activity in 23.8%. **Conclusion:** Our data indicate that
the crude extracts from cerrado's plants inhibited the activity of gelatinases, it could possibly contribute to
the control of degenerative diseases involving the degradation of extracellular matrix.

301 - HIGH ACTIVITY CELLULASES FROM COCONUT SHELL DEGRADING FUNGI

¹Érica D. Albuquerque, ²Fernando Araripe Torres, ³A. Alberto R. Fernandes, and ³Patrícia M. B. Fernandes
¹- Doctoral student - Universidade Federal do Espírito Santo-UFES/RENORBIO, Vitória, ES, Brazil; ²- Research scientist - UNB, Brasília, DF, Brazil; ³- Research scientist - Universidade Federal do Espírito Santo, Vitória, ES, Brazil. e-mail presenting/corresponding author: bioerik@hotmail.com

Cellulases are enzymes that can be used in the food, textiles and biofuels industry, which still represents a high cost. There are several alternatives to reduce their cost, among them the isolation of cellulolytic fungi with high cellulase activity. Agricultural production generates tons of waste per year that must be reused. Hence, the aim of this work was to isolate fungi with high cellulase activity in order to promote the conversion of green coconut shell into fuel. Healthy green coconut shell and coconut shell in decomposition samples were disinfected with alcohol 70% and hypochlorite 10%, washed in sterile distilled water. Small pieces of those samples were plated on PDA culture medium and incubated at 28 °C. Fungi were isolated and purified after 7 days of growth. The initial screening for cellulolytic fungi was performed in solid CMC medium using 0.2% Congo red for staining of the degradation area. The fungi isolates were identified microscopically. For enzyme production, fungi isolates were grown in 1% CMC at a concentration of 10⁷ spores.mL⁻¹. FPase and CMCase activity of the isolates were measured and compared with that of *Trichoderma reesei* RUTC-30 and Celluclast 1.5L derived from *T. reesei* (Novozymes A/S, Denmark). Measurement of cellulase activity was performed by incubation of the substrate with the enzymes in 0.1 M sodium acetate buffer pH 5.0 (2% w/v) at 50 °C for 30 min. DNS reagent was used for reducing sugar determination at 540 nm. The specific activity was calculated as the amount of enzyme required to release 1 µmol of reducing sugar. min. mL⁻¹. Fungi from the genus *Trichoderma* and *Aspergillus* showed a large halo of degradation in solid CMC. The highest FPases and endoglucanases activity, 1.48 FPU.mg⁻¹.h⁻¹ and 1.83 EGU.mg⁻¹.h⁻¹, respectively, was from the coconut shell decompositor fungi *Trichoderma* sp. Cellulolytic activities of the isolates were higher than those of *Trichoderma reesei* RUTC 30 (0.27 FPU.mg⁻¹.h⁻¹ and 0.7 EGU.mg⁻¹.h⁻¹) and that of Celluclast 1.5L (0.037 FPU.mg⁻¹.h⁻¹ and 0.7 EGU.mg⁻¹.h⁻¹). Thus, cellulolytic fungi with high cellulases (endoglucanase e FPase) activity isolated from green coconut shell may be used for conversion of this highly polluting waste in a yeast fermentable source for ethanol production.

1. Solid et al. (2000), IIMC Genomics, 9:327. 2. Eveleigh et al. (2009), Biotechnology for Biofuels, 2:21. Supported by: CAPES, FAPES and PINEP

302 - SURVEY OF MAIN CONTAMINANTS AGENTS IN CELL CULTURE OF CAPRINE SYNOVIAL MEMBRANE

¹Dalva A. A. de Azevedo, ²Mariã Alzira do Carmo Aragão, ³Samilly M. Alves, ³Lauana Borges Santiago, ⁴Francisco Selmo Fernandes Alves, ⁵Raymundo Rinaldo Pinheiro

¹- Graduate student-UVA, Sobral-CE, Brazil; ²- Teacher UVA, Sobral-CE; ³- PhD student-UFC, Fortaleza, Brazil; ⁴- Researcher - Embrapa Sheep and Goats, Sobral-CE; ⁵Leader: Researcher - Embrapa Sheep and Goats, Sobral-CE, Brazil. *Author for correspondence: rizado@cnpq.embrapa.br

The cultivation of animal cells began in the late nineteenth century and over a hundred years has become an important useful technology and successfully in the production of antigens, vaccines and recombinant therapeutic products (Moraes et al. 2007). Contamination by viruses, bacteria, protozoa and fungi is one of the biggest problems in growing cells in vitro (RIZZO et al., 1983). This study aimed to survey the main contaminants in cell culture fibroblasts of goat synovial membrane (MSC). For this, a survey was conducted between June 2004 to June 2010 at the Virology Laboratory in Embrapa Goats and Sheep, Sobral, Ceara state, Brazil. The presence of contamination in bioreactors bottles (cell culture) was observed by macroscopic effects, the presence of hinf in cell maintenance medium (MMC), color change and turbidity, with consequent changes in pH and cell death. These events were concentrated in the first two years (71.4%). For identification of the contaminants, the MMC culture was seeding in Agar Blood and differential media for mycoplasma. The identification carried out by morphological and biochemical procedures. In some cases the mycoplasma contamination were found, however, in fourteen events we found ten bacterial isolates, including *Pseudomonas* spp, *Staphylococcus* spp, Gram positive bacilli and four fungi, those identified by direct observation of their colonies. It is assumed that the contamination may have occurred for the following conditions: water bath, handling equipment, entry of strangers person to working environment and inadequate sterilization of CO2 incubator.

MORAES, ANGELA MARIA; AUGUSTO, F. ELISABETH PIRES, Castilho, Leda R. Technology of cultivation of animal cells: from biopharmaceuticals to gene therapy. São Paulo: Roca. 2007. p. RIZZO, EDDA; TUCHIYA, N. HIROKO; MARTINEZ, HELEN CLELIA. Basic techniques in cell culture - Sao Paulo, Butantan Institute, 1983.

303 - EMERGENCE OF PLASMID-MEDIATED QUINOLONE RESISTANCE DETERMINANTS AAC(6')-IB-CR AMONG ENTEROBACTERIAL ISOLATES FROM OUTPATIENTS IN BRAZIL

Thais V. Podestá¹, Marília C. Franco², and Luciene A. R. Minarini³

¹- Graduate student - UNIFAL, MG; ²- Research scientist - UNIFAL, MG; ³- Research scientist - UNIFAL, MG

Bacterial resistance to fluoroquinolones result from mutations in the quinolone resistance-determining regions of the drug targets, overexpression of efflux pumps, and/or the more recently identified plasmid-mediated low-level resistance mechanisms. *aac(6')-Ib-cr* is a plasmid-mediated quinolone resistance determinant that encodes a variant aminoglycoside acetyltransferase with two amino acid alterations allowing it to inactivate ciprofloxacin through the acetylation of its piperazinyl substituent. We have studied by PCR and DNA sequencing the presence of the *aac(6')-Ib-cr* among nalidixic acid-resistant enterobacterial strains isolated from outpatients from Southeast Brazil collected between January 2000 and May 2005. Antibiotic susceptibility was determined using the standard disk-diffusion and agar dilution methods. The production of ESBL was detected according to Clinical and Laboratory Standards Institute (CLSI) criteria. Among the 257 enterobacterial isolates, *aac(6')-Ib* were detected in 42 (16.3%), comprising *Escherichia coli* and 1 *Klebsiella pneumoniae* *aac(6')-Ib-cr* positive. These isolates were extended-spectrum beta-lactamase-negative. The ciprofloxacin MICs ranged from 0.25 to 128 mg/ml. The results