

# MICRO BIOTEC'11

BRAGA  
01 DEC - 03 DEC

PORTUGUESE SOCIETY FOR MICROBIOLOGY  
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Sociedade Portuguesa de Microbiologia



MATR allelic profiles of  
-RFLP [1]. MATR-VNTR  
stability and discriminatory

64. Célia Leão is a recipient of a

## cell death caused

MA - Department of

nd increased lifespan. In  
most common cause of  
cancer compounds. In a  
nd to inhibit proliferation  
te JNK and to modulate  
dy, we tested the ability  
l modulate autophagy in  
e phytochemicals on cell  
and starvation medium.  
Contrary to our previous  
ctedly, OA was a more  
ensitized HepG2 cells to  
complete medium to 3.5  
markers LC3-II and p62,  
all death induced by OA  
all, the results seem to  
methyl- $\beta$ -cyclodextrin (a  
cell death. In conclusion,  
OA, in particular during  
In the future OA can be  
ns.

CT research grant NaturAge -  
n from the European Community

Higiene e Medicina  
Microbiológicos, Faculdade

the world. Since 1993,  
nosis. High precipitation  
reservoirs of leptospire),  
val in nature. The high  
d human cases and the  
and development of a  
" under USA Scientific  
r 2004 and 2008, was  
iii) laboratory diagnosis,  
: IHMT, to local hospitals  
es and practices of the  
to evaluate the current  
red on LRL databases  
2 sera, received at LRL  
clinical suspicion, were  
Test (MAT) in order to

detect anti-*Leptospira interrogans* sensu lato (s.l.) antibodies. Serological positive results obtained by MAT showed the following distribution: from 2008 to 2010, 60<sup>+</sup>/226 (27%); from 2004 to 2007, 148<sup>+</sup>/370 (40%), and from 1993 to 2003, 248<sup>+</sup>/586 (42%). These findings reveal an important impact of the project's involvement area since the number of cases of Leptospirosis significantly decreased during and after the project's completion. It is assumed that this important reduction is a consequence of the mapping of the major *Leptospira* transmission risk-areas to humans, and the creation of laboratory facilities, namely the diagnosis implementation by a serological screening and PCR technique, avoiding the extreme clinical pictures. Globally, these results promote a continuous and active surveillance in Azores.

PS3: 41

## Incorporation of antimicrobial peptide into bacterial cellulose produced through food waste

**Angela Faustino Jozala<sup>1</sup>, Carolina Alves dos Santos<sup>2</sup>, Renata Aparecida Nedel Pértile<sup>3</sup>, Francisco Miguel Gama<sup>4</sup>, Adalberto Pessor Jr.<sup>1</sup>**

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The incorporation of antimicrobial, such as nisin, or other drugs in bacterial cellulose has a wide applicability in pharmaceutical, medical, chemical, cosmetic, food and other areas. Nisin is a natural antimicrobial peptide used as food preservative; being effective at controlling a broad range of gram-positive bacteria, including the multidrug-resistant pathogen. Bacterial cellulose (BC) is an extracellular polysaccharide produced by strains of *G. xylinus*. BC production using waste as culture media is a novelty, stimulating scale-up for industrial production and extended applications in medical devices. For this reason, the objective of this work was evaluated nisin activity after incorporation into BC standard and produced using waste. For BC production, the following culture media were employed (i) Hestrin and Schramm (standard); (ii) Waste fruits, collected from the disposal of free markets; (iii) Mixture of the waste fruit and milk whey, under 30 °C for 96 hours in static conditions. BC standard and from waste were submerge in 1mL of nisin (Sigma<sup>®</sup>, 1g contain 2.5% of nisin), at different concentrations 250, 125, 62.5, 31.25, 15.63 and 7.81  $\mu$ g/mL, at phosphate buffer saline (PBS) pH 4.5, sterilized by filtration, 0.22  $\mu$ m. They were incubated at 30°C under 100rpm for 4 hours, followed by protein analysis and nisin activity, which was determined by agar diffusion with *L. sakei* as bioindicator. Results demonstrated that all types of BC have the ability to incorporate nisin after 4 hours. Nisin activity was higher in BC, although only 43% of proteins from the initial solution were transferred into the membrane. The same behavior on nisin incorporation was observed in BC standard and from waste, indicating that waste can be applied on BC production. This work can be considered a profitable alternative, generating high-value products with extended applications and contributing to decreasing disposals in the world.

PS3: 42

## Induction of antioxidant defenses by diterpenic phenolics in human fibroblasts for nutritional applications toward anti-aging interventions

**Ana Cristina Carvalho<sup>1</sup>, Andreia C Gomes<sup>2</sup>, Cristina Pereira-Wilson<sup>2</sup>, Cristovao F Lima<sup>1</sup>**

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Human lifespan is increasing in developed and developing countries, rising considerably the percentage of elderly people. Aging is characterized by the progressive accumulation of molecular damage, which leads to altered cellular functioning, reduced stress tolerance and susceptibility to diseases, such as cancer, neurodegenerative and cardiovascular diseases. Consumption of fruits, vegetables and herbs has been related with health promotion due to their content in bioactive phytochemicals. In a previous work, we have shown the ability of curcumin to induce antioxidant stress responses in human fibroblasts, protecting the cells to a further oxidant challenge, which suggests curcumin's potential in anti-aging interventions [1]. In the present work, we propose to evaluate the ability of the phenolic diterpenes carnosic acid (CA) and carnosol (CS) to induce intracellular antioxidant defenses in normal human skin fibroblasts and relate that with anti-aging effects. The ability of these compounds to induce cellular antioxidant defenses was investigated by western blot and biochemical determinations. We observed that human fibroblasts are more susceptible to CS than CA. Carnosic acid was not toxic up to 40  $\mu$ M (the highest concentration tested), CS induced toxicity in human fibroblasts only at 40  $\mu$ M. Both CA and CS at non-toxic concentrations induced significantly glutathione levels, an important intracellular antioxidant, in a concentration dependent manner. Both compounds were also able to induce cytoprotective enzymes such as HO-1, NQO1 and Hsp70, assessed by western blot. We also tested the capacity of these compounds to afford a cytoprotective action by pre-incubating cells with CA and CS before a further oxidant challenge



with *tert*-butyl hydroperoxide (*tert*-BOOH). In conclusion, the induction of antioxidant defenses in human skin fibroblasts by CA and CS may be applied for nutritional applications toward cytoprotective and anti-aging interventions.

[1] Lima et al., *Mol. Nutr. Food Res.* 2011, 55: 430-42. *Acknowledgements:* ACC is supported by BI1-PTDC/QUI-BIQ/101392/2008 grant. This work is supported by FCT research grant NaturAge – PTDC/QUI-BIQ/101392/2008, which is co-funded by the program COMPETE from QREN with co-participation from the European Community fund FEDER.

PS3: 43

### Induction of antioxidant defenses in human HepG2 cells by a methanolic extract of *Hypericum perforatum* cells elicited with *Agrobacterium tumefaciens*

**Cristovao F. Lima, Ana Cristina Carvalho, Gregory Franklin, Alberto C.P. Dias**

CITAB - Department of Biology, University of Minho, Portugal

*Hypericum perforatum* (HP) commonly known as St. John's wort is an important medicinal plant traditionally used in several ailments including mild to moderate depression. In a recent study, elicitation of HP cells (culture of cells in suspension) with *Agrobacterium tumefaciens* (AT) increased significantly the production of compounds with antioxidant and antimicrobial properties [1]. The present investigation has been conducted to test the efficacy of methanolic extracts of elicited and control HP cells in protecting human hepatocytes (HepG2 cell line) upon oxidative stress. Briefly, HepG2 cells were treated with *tert*-butyl hydroperoxide (*tert*-BOOH) to induce oxidative stress, and the ability of the HP extracts to protect against cell death were measured by MTT and LDH leakage assays. The ability of these extracts to induce cellular antioxidant defenses were investigated by western blot and biochemical determinations. When HepG2 cells were co-incubated with 800  $\mu$ M *tert*-BOOH and HP extracts (80  $\mu$ g/ml), only the extract from the elicited HP cells was able to significantly prevent *tert*-BOOH-induced cell death. The extract from control HP cells did not show any protective effect, on the contrary, stimulated *tert*-BOOH toxicity. When HepG2 cells were pre-incubated for 5 h with HP extracts, followed by a period of 16 h of recovery with fresh medium, prior to incubation with *tert*-BOOH, only the HP extract from elicited cells significantly protected against cell death. This suggested the ability of HP extract from elicited cells to induce intracellular antioxidant defenses in HepG2 cells. That was confirmed by the induction of about 40% in the content of glutathione in HepG2 cells, whereas control extract increased only 10%. As well, only the extract from elicited HP cells were able to induce the levels of cytoprotective enzymes such as HO-1 and NQO1. In conclusion, we observed that elicitation of HP cells with AT produced bioactive compounds present in the methanolic extract able to protect HepG2 cells against oxidative stress, and also to induce intracellular antioxidant defenses of this human cell line.

[1] Franklin et al., *Phytochemistry*, 2009, 70: 60-8. ACC is supported by BI1-PTDC/QUI-BIQ/101392/2008 grant. This work is supported by FCT research grants NaturAge – PTDC/QUI-BIQ/101392/2008 and (HyperFood)-PTDC/AGR-ALI/105169/2008, co-funded by the program COMPETE from QREN with co-participation from the European Community fund FEDER.

PS3: 44

### Effect of algae and plant lectins against bacterial biofilm formation

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Biofilms are composed by microbial cells that are irreversibly associated with a surface and are enclosed in a matrix of polymeric material. The search for potential phytochemicals as anti-biofilm agents has become an active area of research. Lectins are sugar binding proteins of non immune origin that agglutinate cells and/or precipitate glycoconjugate molecules. Due to their capacity to bind and recognize specific carbohydrates, lectins can be a potent tool against biofilms. Thus, this work aims to evaluate, *in vitro*, the activity of a set of plant and red algae lectins against clinical relevant bacteria, *Staphylococcus aureus* and *Klebsiella oxytoca*, by the assessment of their capacity to interfere on biofilm formation. Lectins were added to bacteria ( $2 \times 10^6$  CFU/mL) on the moment of biofilm formation in concentrations ranging from 25 to 250  $\mu$ g/mL. Subsequently, the resultant biofilms (48 h) were analyzed in terms of biomass by crystal violet staining and in terms of cell viability by assessing the number of colony forming units. Additionally, the effect of lectins on planktonic growth was also assessed following the optical density of the bacterial cultures along time. Although both groups of lectins were able to reduce the growth of *S. aureus* and *K. oxytoca*, the plant lectins from *Vatairea macrocarpa* (VML) and *Cratylia floribunda* (CFL) showed the better activities. It should be highlighted that VML at 250  $\mu$ g/mL reduced around 90% the planktonic growth