Ethanol extract of *Portulaca oleracea* L. reverts the profile of cell death induced by acetic acid in *Saccharomyces cerevisiae*

Sofia de Jesus [1], **Isabel Alves-Pereira**[1,2], **Rui Machado**[1,3] and **Rui Ferreira**[1,2]

[1] ICAAM Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Universidade de Évora, Núcleo da Mitra, Apartado 94, 7002-554 Évora, Portugal
[2]Departmento de Química, Escola de Ciências e Tecnologia, Universidade de Évora, R. Romão Ramalho, 59, 7000-671 Évora, Portugal
[3]Departmento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Núcleo da Mitra, 7000-083 Évora, Portugal

Saccharomyces cerevisiae exhibits stress response mechanisms that are in many cases identical to those of higher eukaryotes. Tests to evaluate the oxidant/antioxidant power of numerous chemicals including the detection of changes in cell growth and cell viability as well as enzymatic and non-enzyme antioxidant response, are increasingly being used, because their results are reproducible and, in many cases, easily applied to the man.

Acetic acid, a by-product of alcoholic fermentation in several species of yeast is described as an oxidizing agent, able to induce oxidative stress and cell death. In *Saccaromyces cerevisiae*, the cell growth is often inhibited by weak acids such as acetic acid when is associated with high levels of ethanol and other toxic metabolites which, block fermentative metabolism by feedback mechanisms. To counteract the effects of acetic acid, *S. cerevisae* has metabolic resources that allow it to avoid its accumulation at high and potentially toxic levels inside the cell. Although in the absence of other carbon sources, acetic acid can be metabolized by gluconeogenesis, this preventive mechanism is repressed in the presence of glucose, so its accumulation becomes easier, behaving as an inducer of programmed cell death for exposure level of 20-80 mM acetic acid.

Purslane (*Portulaca oleracea* L.) is a green leafy vegetable whose stems and leaves are edible and frequently used in culinary. The abundance and diversity of phytochemicals with biological activity detected in this plant contribute to its high functional value. Although nitrogen is the nutrient that most influences its production, it can decreases its functional value when applied in excess. This requires that the nitrogen fertilization of the plant is in the amount strictly necessary to ensure reasonable productivity without environmental impact.

The main purpose of this work was to evaluate the biological effects of leaf-blade extracts of *Portulaca oleracea* L. var. sativa, fertilized with NH_4NO_3 (60 kg/ha) in *S. cerevisiae* UE-ME_3 exposed to 25 mM acetic acid.

The simultaneous exposure of *S. cerevisiae* UE-ME_3 to 25 mM acetic acid and 12% ethanol extract of leaf-blade purslane, with high total phenols content and antioxidant power, during 200 min, maintained the biomass produced (dry weight), cell viability (cfu), cell damages levels (MDA), oxidative stress marker (GSH/GSSG ratio) and antioxidant enzyme activities ALP, GPx and CTA1 close to the control group, followed by an increase of the catalytic activities GR and SOD1. Ethanol extract of leaf-blade purslane seems to revert the cell death signature induced by 25 mM acetic acid.

Keywords: yeast; oxidative stress, purslane, green leafy vegetable, nitrogen

Acknowledgments
This work is funded by FEDER Funds through the Operational Programme for Competitiveness Factors - COMPETE and National Funds through FCT - Foundation for Science and Technology under the Strategic Projects PEst-C/AGR/UI0115/2011 and PEst-OE/AGR/UI0115/2014.

References

[1] Ludovico, P., Sousa, M., Silva, M., Leão, C. & Côrte-Real, M. (2001). *Saccharomyces cerevisiae* commits to a programmed cell death process in response to acetic acid. Microbiology, 147, 2409-2415.
[2] Oliveira, I. Valentão, P., Lopes, R. Andrade P., Bento, A. & Pereira, J. (2009). Phytochemical characterization and radical scavenging activity of *Portulaca oleracea* L. leaves and stems. Microchemical Journal, 92, 129-134.
[3] Dkhill, M., Moniem, A., Al-Quraishy, S. & Saleh, R. (2011). Antioxidant effect of purslane (*Portulaca oleracea*) and its mechanism of action. Journal of Medicinal Plants Research, 5(9), 1589-1563.
[4] Kitchen, N.R., Goulding, K.W.T & Shanahan, J.F. (2008). Nitrogen in the Environment: Sources, Problems, and Management.J.L. Hatfield and R.F. Follett (Eds). Chapter 15. Proven Practices and Innovative Technologies for On-Farm Crop Nitrogen Management 483-517)

Evaluation of microbiota isolates in human pre and post-pasteurized human milk and feces of infants

Yara N. Correa[1], **Olga I. Montoya**[3], **Alejandra Buitrago**[3]

[1] Master's student Biotechnology. National University of Colombia, Faculty of Sciences. A.A 3840, Medellín Colombia. <yncorreah@unal.edu.co>
[2] Undergraduate student in Biological Engineering, Faculty of Sciences, National University of Colombia. Medellin Colombia. <Jeabuitragova@unal.edu.co>
[3] Associate Professor. National University of Colombia, School of Biosciences, Faculty of Sciences. National University of Colombia, A.A 3840. Medellín, Colombia. <Oimontoy@unal.edu.co>

Breast Milk (LM), with its high nutritional value, provides infants with the necessary elements for a healthy development. It includes the maturation of the immune system, as well as commensal microorganisms, and among them, those with probiotic properties, such as some Bacteria from the Lactic Acid Bacteria (BAL) group. A daily consumption of approximately 800 mL of LM contains up to 107 Colony Forming Units per milliliter (CFU / mL) of commensal bacteria. This is reflected in the fecal microbiota of the healthy infants, so the alteration of the same is related to a proinflammatory state, increased morbidity and mortality, and an increase in the incidence of immune and infectious diseases, such as Necrotic enteritis (NE). In Antioquia, NE's decreased from 3.33 to 0.22% during 2013 to 2015, due to the creation of the Human Milk Bank (BLH) located in the Hospital General of Medellin (HGM). LM is considered a natural alternative to prevent and control infectious pathologies in children under 5 years of age to avoid the increase of resistance to antibiotics, which is progressing more and more. Although the BLH is the only institution of this type in Antioquia, it benefits 95.21% of the population of infants who cannot be breastfed, and 4.79% children of other departments in Colombia. As protocol in the BLH, acid LM is discarde due a high CFU / mL count of viable microorganisms, without verifying whether they are pathogenic, altering or beneficial, such as BAL, in both pre and post-pasteurized milk. To this day, there are no studies that characterize the microbiota present in the LM in Antioquia. Therefore, this research aims to evaluate the microbiota present in pre and post-pasteurized LM and compare it with the microbiota in the feces of the infants and the milk of their mothers.

METHODS: Samples were taken from 100 donors and their 100 infants (0 to 6 months). Those samples were processed in the Laboratory of Water and Food Microbiology of the National University of Colombia, in the respective culture medium (Agar MRS, M17 and TOS); the bacteria were isolated and characterized morphologically and biochemically from the pre and post-pasteurized LM as well as from the feces of the infants, carrying out on each isolate the selective tests to evaluate probiotic potential. RESULTS: We verified the presence of BAL with probiotic potential in both pre and postpasteurized LM and feces of infants. The ones with the highest potential were genus *Lactobacillus* and genus *Bifidobacterium*. **CONCLUSION**: It was demonstrated the correlation of the microbiota of mother and child through the LM. This showed the importance of breastfeeding in correcting the risk factors mentioned above.

Keywords: Micobiota, Probiotics, Maternal milk, Feces.