Aluno: Ana Isabel Teixeira Carvalheira

Orientador: Doutora Paula Teixeira

Tema da Tese: *Acinetobacter* and public health: risks posed by strains isolated from community and hospital

Acinetobacter spp. are ubiquitously distributed in nature. In the past, these microorganisms were considered saprophytes of little clinical importance. The occurrence of nosocomial infections caused by *Acinetobacter* spp., mainly *A. baumannii*, has increased rapidly and many outbreaks have been reported worldwide representing an important public health issue. There have been reports of panresistant *A. baumannii*, which are resistant to every marketed antibiotic.

Several studies have been published on the occurrence of *Acinetobacter* spp. in clinical specimens and hospital environments, with the main focus on a restricted group of species within this genus (namely *A. calcoaceticus – A. baumannii* complex*)*. However, information about environmental and food sources of the different species of this organism is scarce, and the mechanisms of transmission and spread between community and hospital have not been explored.

The global objective is to identify/characterize *Acinetobacter* spp. from both community and hospital. The tasks include: 1) establishment of a project culture collection; 2) evaluation of the prevalence of *Acinetobacter* spp. inside and outside hospital; 3) identification and genotypic characterisation of *Acinetobacter* spp.; 4) evaluation of antibiotics resistance and mechanisms of resistance of *Acinetobacter* spp.; 5) evaluation of capacity of *Acinetobacter* spp. to adhere to epithelial cells; 6) evaluation of capacity of *Acinetobacter* spp. to form biofilms and the activity of disinfectants/antiseptics against planktonic and biofilm cultures.

Two different pre-enrichment media (Baumann Enrichement Medium and Acetate Minimal Medium) and two selective agar media (Leeds Acinetobacter Medium and Chromagar Acinetobacter) were evaluated in relation to their ability to improve the isolation of *Acinetobacter* spp. from food samples. Combination of Acetate Minimal Medium enrichment followed by isolation on Chromagar Acinetobacter showed to enhanced *Acinetobacter* spp. detection. This method was selected to test the presence of these organisms in 17 samples of vegetables, including: lettuce, tomato, parsley and carrots. All samples showed contamination by Acinetobacter. The genus of the collected isolates was further confirmed by phenotypic and

genotypic tests. Isolates proven to be *Acinetobacter* were typed by REP-PCR (Repetitive Extragenic Palindromic sequence-based - Polymerase Chain Reaction). Isolates presenting different typing patterns were selected for molecular identification of *Acinetobacter* species by *rpoB* gene sequencing.

Aluno: Ana Lúcia da Silva Oliveira

Orientador: Doutora Manuela Pintado

Tema da Tese: Preservation of nutritional and functional properties of fresh and processed fruit with emphasis on phytochemicals

Fruit are an essential part of a healthy diet and vehicle of a number of unique health promoting phytochemicals. Being very perishable, fruit require preservation, both as fresh or processed foods. From nutritional and functional point of view is important to understand the consequences of processing in food composition in order to select the best technological conditions required for the preservation of fruit health benefits. Processing of fruits may entail different phases that naturally affect the nutritional and functional quality of the final product.

The general objective of this Ph.D. programme is to understand the effect of factors related to storage of fruit and with fruit processing on the nutritive and functional properties of fruits — strawberry and peach. This information will be integrated to optimize processing conditions that maximize the content of beneficial antioxidants compounds aiming the development of functional food with specific properties. It is expected through this project to establish the impact that specific processing conditions, namely postharvest storage, pasteurization, freezing, additives adding and incorporation in food matrix have upon antioxidants compounds naturally present in the fruits.

The results obtained during the first two years of research reveals that processes like pasteurization of fresh fruit and subsequent storage duration have a significant impact in phytochemicals namely anthocyanins in strawberry and carotenoids in peach. Fruit pH values variation affected significantly anthocyanins in strawberries, which increase for lower pH values; carotenoids from peach revealed a higher degradation at lower (2.5) and higher (4.5) pH values tested. Storage temperature also proved to be important, since lower temperature preserved better functional compounds than room temperature, as expected.

When an industrial process was assessed it revealed that ingredientation leads toan increase of compounds extraction and the pasteurization had significant impact only for strawberry. In both fruits storage duration promoted decreases, but most significantly in anthocyanins.

The effect of modified atmosphere (gas mixtures: 10% oxygen+90% nitrogen, 100% nitrogen and air) during strawberry and peach storage at 4 and 23 ºC demonstrated a lower decrease in anthocyanins and in peach samples for the carotenoids, β-cryptoxanthin and β-carotene, at 100% N2.

The effect of incorporation in food matrix was studied in yoghurt throughout normal storage time, and interaction between fruit polyphenols and carotenoids and matrix components (mainly proteins) were established. After fruit addition and during storage period it was observed a decrease in antioxidant capacity, total phenolics as well anthocyanins from strawberry preparates. Soluble proteins from milk, β-lactoglobulin and α-lactalbumin also decreased.

Contrarily, incorporation of peach preparates in yogurts showed a decrease only for carotenoids in the end of storage time and a slight decrease in proteins.

Based on the result obtained as future work we intend to perform some experiments with pure proteins and fruit preparates in order to get a better understanding about protein-functional compound interaction. Currently, we are analyzing results obtained for each functional marker as affected by several processing variables, in order to establish a mathematical model to predict functional markers depreciation according with the process applied. Finally, a study encompassing emerging technologies that could be applied to fruit to minimize polyphenols and carotenoids losses during manufacturing processing is planned.

Aluno: Ana Rita Lado Teixeira Ribeiro

Orientador: Doutora Paula Maria Lima e Castro

Tema da Tese: Chiral pharmaceuticals in the environment: enantiomeric ratio and biodegradation studies

Foi desenvolvido um método de HPLC-FD para quantificar simultaneamente os enantiómeros da fluoxetina e do seu metabolito norfluoxetina, usando uma coluna quiral Chirobiotic™ V, 5µm (15 x 0.46 cm ID) e uma fase móvel constituída por etanol e acetato de amónia 10mM. Este método foi aplicado a ensaios de biodegradação da fluoxetina racémica, utilizando meio mínimo suplementado com fluoxetina e inoculado com lamas ativadas ou com uma cultura pura (Labrys portucalensis). Os resultados mostraram que esta cultura é capaz de remover em maior extensão o enantiómero (*R*)-fluoxetina. Foi também realizado um estudo da biodegradação dos enantiómeros puros da fluoxetina pela mesma cultura.

O método desenvolvido foi adaptado ao estudo de amostras reais, tendo sido desenvolvido um método de extração em fase sólida (SPE, Solid Phase Extraction) para remover interferentes e concentrar a amostra. Foram testados vários tipos de cartuxos: fase reversa com equílibrio hidrofílico-lipofílico (Oasis HLB), fase reversa trocador de catiões em modo misto para bases (Oasis MCX), fase reversa trocador de catiões fraco em modo misto para bases fortes (Oasis WCX) e polímeros molercularmente impressos (Supelco MIP). Os cartuxos que mostraram ter melhor recuperação foram os Oasis MCX. Este método foi aplicado em estudos de biodegradação, utilizando amostras de efluente de uma Estação de Tratamento de Águas Residuais suplementadas com fluoxetina racémica. FLX residual foi detectada até ao 46º dia, contudo a degradação não foi enantioselectiva e não se verificou a formação do metabolito NFLX.

Está a ser desenvolvido um método de LC-MS para determinar a fração enantiomérica de vários fármacos quirais e um metabolito em amostras ambientais: alprenolol, metoprolol, salbutamol, bisoprolol, ciatalopram, venlafaxina e norfluoxetina, além dos fármacos inicialmente propostos atenolol, propranolol e fluoxetina. O método de separação cromatográfico foi optimizado usando uma coluna quiral Chirobiotic™ V, 5µm (15 x 0.21 cm ID) e uma fase móvel constituída por etanol e acetato de amónia 10mM (fluxo 0.32 mL min^{-1}).

Aluno: Ana Rita Varela

Orientador: Doutora Célia Manaia

Tema da Tese: Tracking antibiotic resistance from hospital effluents to the surrounding environment

The discharge of untreated hospital effluents has been associated with the dissemination of antibiotic resistant bacteria into the environment. However, studies on their impact on the receiving urban wastewater treatment plants (UWTP) are scarce. The main goal of this study was to compare the loads of antimicrobials (antibiotic and/or heavy metals), antibiotic resistance prevalence and bacterial community structure (based on 16S rRNA gene PCR-DGGE analysis) of a hospital effluent with those of the receiving UWTP. Antibiotic resistance and antimicrobial residues loads were assessed through successive sampling campaigns of raw hospital effluent and raw and treated wastewater of the UWTP.

The bacterial communities of hospital effluent and raw inflow of the UWTP were distinct, although with higher similarity between them than with the treated outflow. The percentage of antimicrobial resistance detected in the hospital effluent was generally higher than in the raw inflow and treated effluent of the UWTP. Variations on the bacterial community structure in wastewater were correlated with the concentration of antimicrobials and with antibiotic resistance prevalence. Antibiotic resistance in the urban wastewater treatment plant may have other sources than the hospital effluent, but its contribution cannot be neglected.

Aluno: Carla Sofia Sancho dos Santos

Orientador/Co-Orientador: Doutora Marta Vasconcelos / Prof. Doutor António Osmaro S. S. Rangel, Doutora Susana Carvalho

Tema da Tese: IDC: Unravelling the Molecular and Physiological Components that Contribute to Iron Deficiency Chlorosis

Iron Deficiency Chlorosis (IDC) is a serious environmental problem affecting the growth of several crops in the world. Legumes, such as soybean (*Glycine max* L.), are particularly susceptible to IDC when grown in alkaline soils. Several strategies have been attempted in order to prevent and correct iron (Fe) deficiency in plants, but these are not economically feasible. Cultivar selection remains the most practical measure for avoiding IDC, but the morphological and molecular mechanisms behind contrasting cultivar responses to iron deficiency are not fully understood. Plants have been classified as 'Fe-efficient' if they respond to Fe deficiency stress by inducing biochemical reactions that make Fe available in the soil and 'Fe-inefficient' if they do not. In this study, an Fe-efficient (PI 437929) and an Fe-inefficient (PI 378676) *G. max* accession, obtained from GRIN (Germplasm Resources Information Network, USA), were grown at 0 and 20 μM Fe (III)-EDDHA, to better clarify the mechanisms involved in this variant efficiency.

The development of IDC symptoms, e.g. yellowing of leaves and stunted growth, was evaluated 10 days after transferring to Fe-sufficient and Fe-deficient hydroponic conditions. It was shown that under Fe deficiency, the Fe-inefficient accession had 37% lower chlorophyll content (SPAD values), produced 31% longer roots and 29% smaller shoots, resulting in stunted growth and smaller leaf area as compared to the Fe-efficient accession. Dicotyledonous plants such as *G. max* utilize a membrane-bound ferric chelate reductase that reduces ferric iron (Fe^{3+}) to the more soluble ferrous form (Fe^{2+}) and the activity of this enzyme was quantified. The Fe-inefficient accession had significantly higher reductase activity ($P<0.05$) when compared to the Fe-efficient one, possibly to alleviate Fe stress and generate more absorbable Fe^{2+}. The mineral content was also evaluated using ICP-OES.

Additionally, to gain insight on the molecular mechanisms involved in Fe-uptake, an SSH (Suppression Subtractive Hybridization) cDNA library from roots of both accessions grown in Fe deficiency is underway.

Future work will focus on evaluating the effectiveness of new chelating agents in enhancing Fe uptake and, therefore, reducing IDC symptoms.

Aluno: Dália Rosa Alves Carvalho

Orientador: Susana Carvalho / Marta Vasconcelos, Ep Heuvelink

Tema da Tese: Understanding and enhancing potential vase life of Rosa hybrida: from pre-harvest conditions to post-harvest longevity

Water stress is one of the most common post-harvest quality problems, resulting in shorter vase life of cut flowers. The main objective of this thesis is to understand the physiological and genetic mechanisms involved in the excessive water loss in rose plants developed under high relative air humidity (RH) (> 85%). Previous studies have shown that high RH leads to stomatal malfunctioning, but the reasons behind that problem and possible ways to solve it are not yet known. In this study, two strategies have been tested to improve stomatal functionality (1) increased air movement and (2) increased salinity in the irrigation solution.

Firstly, it has been tested the hypothesis that increased air movement during leaf expansion would stimulate the production of abscisic acid (ABA), a stress hormone involved in the stomatal closure, leading to a better stomatal functioning. For this purpose, plants were grown under moderate (60%) and high (90%) RH combined with no air movement or with a continuous wind speed of 0.92 ± 0.033 m s^{-1} throughout leaflet development. Although air movement improved desiccation tolerance of leaves grown at high RH (from 21% to 40% RWC – relative water content – after 4 h of leaflet desiccation), the RWC of leaflets grown at moderate RH was significantly higher (78%). The lack of a significant increase in the ABA concentration at high RH might explain the still poor low stomatal functioning when compared to moderate RH.

Secondly, the hypothesis that increasing the salinity level (EC) of the irrigation solution (2, 4 and 6 dS m^{-1}) throughout plant development can enhance stomatal responsiveness to closing stimuli in high RH-grown plants was tested. It was found that in leaflets developed at high RH, the relative water content (RWC) after 4 h of desiccation increased from 18% (EC 2, control) to 42% (EC 6). In addition, it was shown that the nocturnal stomatal conductance (g_s) of intact leaflets from high RH-grown plants was linearly reduced with increased salinity levels (being 320 mmol $m^{-2}s^{-1}$ at EC 2 and 130 mmol $m^{-2}s^{-1}$ at EC 6). Nonetheless, the levels achieved of RWC and nocturnal g_s were still significantly different from the ones observed in moderate RH-grown plants grown at EC 2. It was concluded that increased salinity within the studied range can partly counteract the negative effect of high RH on stomatal functioning. However, this strategy should be carefully applied to roses as although no leaf chlorosis (due to salt toxicity) was observed in the first flush, after harvesting the flower stalk, severe symptoms appeared on older leaves, especially at EC 6.

Last year, the assay from the effect of the air movement on the stomatal responsiveness was presented. This year, the focus will be on the most recent work aiming at understanding the influence of salinity on stomata responsiveness.

In the future, gene expression related to stomatal responsiveness in plants grown under contrasting RH levels will be studied in a segregating tetraploid cut rose population.

Aluno: Helena Maria Gomes Moreira

Orientador: Paula Maria Lima Castro Co-*Orientador:* António Osmaro Santos Silva Rangel

Tema da Tese: Investigations into the role of soil microorganisms in the re-vegetation of environmentally disturbed sites as a tool for land use restoration and biomass production

Abandoned industrial and mining activity left a legacy of environmentally disturbed sites. The establishment of a vegetation cover reducing contamination namely by metals, mobility and/or bioavailability for entry into the food chain is a potential economically feasible and environmental friendly alternative. The use of plant species with high biomass yields allows not only to revert degraded soil, but also the use of the biomass produced for other activities from which economical gains can be achieved, such as energy production, avoiding the destruction of natural habitats by conversion into farmland.

Plant growth promoting rhizobacteria (PGPR) communities can play a major role in the establishment of plants and have been used to reduce plant stress associated with phytoremediation of contaminated soils. Arbuscular mycorrhizal fungi (AMF) are also a major component of the rhizosphere and form mutualistic associations with most plant species, promoting plant growth and greatly influencing their survival.

The main objectives drawn in this working programme are: selection of plant species that inhabit heavy metal contaminated sites with known biomass production potential and plants with highs levels of metal accumulation which may help the stabilization of metal polluted soils; isolation of AMF from colonized plants; selection of some PGPR and screening of their plant growth promotion abilities; evaluation of the contribution of the PGPR and AMF in the establishment of selected plant species in disturbed soils.

The experimental work began with a survey of the colonizing species proliferating in an industrialized heavy metal contaminated site – Estarreja. In a 1-year screening, 27 species were found with some plants presenting high Zn tissue accumulation and being potentially suitable for phytoextraction and phytostabilisation. *Zea mays* was one of the chosen plants to proceed the investigation, due to its energetic valorization potential. In greenhouse experiments, maize plants were grown in Cd and Zn contaminated soils and were inoculated with two PGPB - *Ralstonia eutropha* and *Chryseobacterium humi.* At the end of the experiment plants were harvested and levels of Zn and Cd in their roots and shoots were determined. Biomass and metal accumulation were assessed and the bacterial dynamics evaluated. Along with these bacteria, three other PGPR, *Pseudomonas fluorescens*, *Agrobacterium tumefaciens* and *Pseudomonas reactans* are being evaluated for their plant-growth features and their abilities to improve plant health in heavy metal contaminated soil. Two of these bacteria will be then selected and co-inoculated with two AMF fungi in maize plants in a natural contaminated soil. Extrapolations will be made to field scale in order to assess their real applicability and possible gains brought by their application in further activities such as biofuel production.

Aluno: Inês Carvalho dos Santos

Orientador: Prof. Doutor António O. S. S. Rangel

Co-Orientador: Doutora Raquel Mesquita

Tema da Tese: Development of automatic microflow systems for monitoring microbiogical, biochemical and physico-chemical parameters in bathing waters

The quality of bathing waters must be considered an issue of public health. In fact, the first European regulation of bathing waters dates from 1975 when the 'Bathing Water Directive' was created. In Europe, several countries are dependent on their coastal regions due to the economical impact of tourism and marine recreation. Portugal accounts for about 2.5% of the reported bathing waters of the European Union with a total of 540 bathing waters to be monitored of which 443 were coastal bathing waters (417 on sea; 26 on estuaries) and 97 freshwater bathing waters (87 on rivers; 10 on lakes). Although the main concern is the presence of fecal coliforms, namely E. coli, other microbiological parameters (faecal streptococci, salmonella and entero viruses) and physico-chemical parameters such as phenols, ammonia, heavy metals and nutrients (nitrates and phosphates) are also quite important to be assessed.

The current methods of analysis are quite laborious and time consuming, so the development of expedite automated methodologies for accurate, real time analysis is essential. Flow analysis methods, namely sequential injection analysis (SIA), meet these requirements and also present the extra advantage of low reagent consumption and effluent production. SIA is based on a multi-position valve and uses programmable flow to mix and transport samples and reagents to the detector. Nevertheless, when biochemical and microbiological assays are concerned, a further miniaturization is desirable. This can be achieved by the recently developed microSI-LOV approach which is based on an integrated manifold microfabricated onto a selector valve. It includes a micro flow cell, in which light is transported via fiber optics cables, and eventually directed to a miniaturized charge coupled device detector. Micro assays can be performed since all elements of the manifold are centralized minimizing the flow path, especially important for reagent based biochemical assays. One of the main features of microSIA, distinguishing it from the nano-scale or micrototal analysis systems, is the capability to use high volume-to-conduit surface ratio which minimizes the surface adsorption problem. Additionally, biochemical reactions and separation processes can be carried out in microbeads, thus providing renewable sensing surfaces (bead injection).

The main objective of this PhD project is to make a contribution to the development of real-time, cost-effective, automatic and miniaturized sensing systems to monitor the quality of bathing waters. The systems to be developed will hopefully provide a closer insight on aquatic ecosystems and biogeochemical studies and enable to monitor biological responses to induced environmental changes.

To achieve the above mentioned purpose, we propose to develop flow techniques based on the microSI-LOV concept, associated to the bead injection technique, to automate biochemical and microbiological assays. In fact, the lab-on-valve seems to be the most appropriate choice for

biochemical assays due to the possibility of handling small amounts of fluids (i.e. microliters). With this approach, the determination of enzymatic activity, a reliable biomarker of microorganisms presence, can be carried out with a minimal consumption of reagents and standards (along the guidelines of green chemistry). A combination of this miniaturized system with the bead injection approach is also envisaged for its application to immunoassays, facilitating microbiological analysis namely of faecal coliforms (E. coli). These microbiological parameters are essential quality requirements for bathing waters.

Furthermore, the possibility of making multiparametric analysis will be explored. The aim is to develop novel methods for the determination of nutrients (nitrate and phosphate), ammonium, phenols and surface-active substances. These parameters, although less pertinent than the microbiological parameters, are also crucial for the overall quality status of bathing waters.

Taking advantage of sampling campaigns programmed on the basis of the on-going FCT UV filters project, these methods will be applied to bathing waters (inland and costal) in Northwestern Portugal and the results compared with classical reference methods.

Aluno: Joana Inês Bastos Barbosa

Orientador: Paula Cristina Maia Teixeira

Tema da Tese: Production of orange juice powder, with a probiotic culture incorporated, by spray-drying

Objective

The overproduction of orange fruit in Portugal is an economic cause of concern. The main idea of this study is to develop an alternative to solve that kind of problems, using the huge amount of oranges to make and preserve orange juice by spray-drying.

Probiotic bacteria such as lactic acid bacteria (LAB) have been linked to several health benefits to humans, especially in the prevention of intestinal disorders. The production of a natural product, such as orange juice, would be more valuable with a probiotic culture incorporated with LAB.

The objective of this study involves the production of an orange juice powder by spray-drying, containing a probiotic (or group of probiotic) bacteria; understanding of the stress response of probiotic bacteria to the spray-drying and its relation with the functional properties.

Results

The optimization of the spray-drying conditions (parameters such as inlet and outlet air temperatures and feed flow rate) allowed the achievement of a maximum income of orange juice powder.

Conditions to obtain the maximum survival of the probiotic bacteria in orange juice powder are still being investigated.

Future Work

The effect of sub-lethal stresses such as heat shock, osmotic shock, pH and oxidative stresses for each probiotic bacteria will be next investigated, in order to know if some stress allow the increase of probiotic bacteria survival after spray-drying and during storage.

Aluno: Joana de Freitas Salgado do Fundo

Orientador: Cristina L.M. Silva/ Mafalda A.C. Quintas

Tema da Tese: *A systematic approach to the study of physical properties and stability in food systems – the relationship with molecular mobility.*

Food physical properties are critical for product and process design, safety and sensorial attributes. Food scientists often collide with difficulties in comparing data obtained with different experimental methods, and few studies compare data from different food systems. As a result, understanding the relation molecular constituents- structure -function is still in its first steps.

The main objective of this project is to contribute to clarify the influence of mobility on the physical properties of food systems. For that, chitosan/ glycerol films (with different chitosan/ glycerol and water concentrations) and fresh-cut fruits (pear and melon) were used as samples. Micro and macroscopic behaviour was analyzed, by means of assessing texture, dynamic linear viscoelastic behaviour and thermodynamic transitions. Molecular mobility is measured by means of Nuclear Magnetic Resonance.

Another important goal of this project is to identify the "baseline" mobility for stability in high water content food products, therefore, comparing the molecular mobility and quality factors of fresh-cut pear and melon, along shelf-life, is being carried out. This is important because, due to fruits cellular structure, water can be present in both intra and extra cellular spaces and this influences the behavior of water mobility.

In relation to chitosan / glycerol films experiments it could be concluded that molecular mobility contributed to the understanding of the films molecular rearrangements. NMR measurements showed two different behaviours for the two components analysed, water and glycerol: while glycerol is mainly bounded to the chitosan chain network, the water present in the system is predominantly free from the polymeric chain. However, it was possible to infer that for lower glycerol concentrations, free chitosan binding sites can also be occupied by water molecules. Water content and water activity measurements also allowed to conclude that not only the water content affects the water mobility, but also structural differences in the film may influence the water relaxation times. Moreover, water mobility relates to the water in the bulk and thus complements information on water activity of a system. Another important result is that, at room temperature, molecular mobility decreases while glass transition temperature increases, according to classic polymer theory. The crystallinity increases with increasing water and glycerol mobility, showing that once the polymeric chains are organized in the crystalline lattice, the interaction polymer/ plasticisant is minimised, the free volume of the system increases and the water and glycerol molecules are thus free to move in the matrix.

Results from fresh-cut fruits experiments are still being analysed.

As future work the molecular mobility of sucrose solutions will be characterise and compared with macroscopic properties published data.

Aluno: Maria Manuela Faria Amorim

Orientador: Maria Manuela Estevez Pintado

Tema da Tese: Development and Characterization of functional ingrediants through valorization of spent brewer yeast: peptide concentrates and ß-glucans with biological activities.

Spent brewer's yeast (*Saccharomyces cerevisiae*) is a by-product of the brewing process and makes up 2–3% of total beer production. Most of this yeast is sold as animal feed at low price or has to be disposed as waste. However, certain cell components, e.g. the structural cell wall polymer β-glucans possess a bioactivity and thus a potential physiological value.

Those compounds are being referred to as biological response modifiers because of their ability to activate the immune system. β-glucan is believed to have various immunomodulatory properties. Studies *in vitro* and *in vivo* reveal that the immunostimulating activity depends on structure, molecular weight and number of branches. These compounds also exhibit hypocholesterolemic, antioxidant and anticoagulant properties. Recently, they has been demonstrated to be anti-cytotoxic, antimutagenic and anti-tumorogenic, making them promising candidate as pharmacological promoters of health.

In the other hand, biologically active peptides has stepped into the limelight with verified particular interest to food science and nutrition - as they have been shown to play several physiological roles *viz.* opioid, immunostimulating, antimicrobial, antioxidant, anti-hypertensive activities. Hence, these peptides can be obtained from yeast extracts through methods of autolysis, plasmolysis and hydrolysis, but the most frequently manufacturing practice is autolysis. In this process, hydrolytic enzymes, particularly protease and nuclease, break down insoluble macromolecules like proteins and nucleic acids to soluble products of peptides, amino acids (mainly glutamate), nucleotides and amino acid derivates. few studies have exploited the production of peptide hidrolysates of high nutritional value from brewer yeast by autolysis in order to up-grade this by-product from the brewing industry, but the hydrolysis using specifc enzymes to obtain new extracts with bioactive properties of high value added has not been subject of study. That suggests they can be used as a potential source of biologically peptides. Therefore, producing a high-value-added product e.g. functional food from yeast glucan and yeast peptides could benefit breweries and the yeast industry by receiving an additional source of income and eliminating the costs of waste removal.

The general objective of this work is to use the spent brewer yeast through the development, characterization and validation of functional ingredients, in particularly peptide concentrates and β-glucans with biological activities in order to incorporate in new food or feed products.

In this first year, the following specific objectives were achieved i) characterization of by-product of brewering industry (ii) production of peptide concentrates from hydrolysis spent yeasts and obtention of β-glucans (iii) identification of biopeptides by sequencing and physical-chemical characterization of β-glucans. In general, separation and purification procedures of proteins and polysaccharides was established and yeast peptide extracts were obtained after autolysis of the spent brewer yeast, provided by Unicer. Afterwards, the mixture was ultrafiltered with a 10 kDa

cut-off membrane and hydrolyzed with proteases from aqueous extract of *Cynara cardunculus*. Retentate was nanofiltrated with 3 kDa and 1 kDa cut-off membrane. Each of yeast obtained were tested *in vitro* for their antihypertensive activity, demonstrating, depending on the extract, the existence of fractions with excellent activity (IC50 between 50 and 100 g/ml).

Future works: biological activities *in vitro viz.* antimicrobial, antioxidant and pre-biotic activities; biological validation *in vivo* - anti-hipertensive effect of peptide concentrate, antiulcerogenic and anti-inflammatory activity; biological validation *ex vivo* – anticarcinogenic and immunomodulatory activity; application the most active β-glucans and yeast peptide concentrate in selected food matrices.

Aluno: Maria José Gonçalves Alves

Orientador: Prof. Manuela Pintado

Tema da Tese: Espécies bacterianas multi – resistentes do CHTMAD E.P.E – Unidade de Chaves: Perfil de sensibilidade e actividade antimicrobiana de cogumelos silvestres do Nordeste de Portugal**.**

Nas últimas décadas a presença de Microrganismos multirresistentes em infecções hospitalares tornou-se um grave problema de saúde pública. Uma vez que o consumo de antibióticos exerce pressão selectiva na emergência de resistência bacteriana, torna-se pertinente sensibilizar no sentido de monitorizar o seu uso. Por outro lado é amplamente reconhecida a necessidade de desenvolver novos agentes antimicrobianos para minimizar a ameaça de resistência aos diferentes antimicrobianos existentes no mercado. Assim, é nosso propósito avaliar a evolução das multirresistências ao longo de 3 anos em diferentes microrganismos utilizados a nível hospitalar, com o intuito de mostrar o aumento progressivo das resistências e assim sensibilizar a comunidade médica para o combate ao uso indiscriminado de antibióticos, bem como para a implementação de uma política de utilização de antimicrobianos adequada à epidemiologia local. Tendo em consideração a problemática anteriormente referida avaliamos *in vitro* a atividade antimicrobiana de extractos de cogumelos silvestres da região do Nordeste Transmontano sobre diferentes espécies bacterianas multirresistentes, bem como dos diferentes compostos puros. Pela análise dos resultados estatísticos da evolução das multirresistências, detetamos um perfil de resistência em que é evidente o agrupamento de microrganismos. É evidente o aumento das resistências aos β - lactâmicos e às quinolonas por parte dos microrganismos Gram negativos. Verificamos ainda que microrganismos pertencentes à flora normal como o *S. epidermidis* se aproximam do perfil de resistência de microrganismos multirresistentes como MRSA. Quanto à avaliação da actividade antimicrobiana dos extractos de cogumelos os resultados das Concentrações Mínimas Inibitórias (CMIs) mostraram que os extractos de *Russula delica e Fistulina hepatica* inibem o crescimento das bactérias Gram negativas (*Escherichia coli, Morganella morganni e Pasteurella multocida*) e das Gram positivas (*Staphylococcus aureus, MRSA, Enterococcus faecalis, Listeria monocytogenes, Streptococcus agalactiae e Streptococcus pyogenes*). Um efeito bactericida de ambos os extratos foi observado para a *Pasteurella multocida, Streptococcus agalactiae* e *Streptococcus pyogenes* com Concentrações Mínimas Batericidas (CMBs) de 20, 10 e 5 mg/mL, respectivamente. *Lepista nuda* demonstrou efeito bactericida sobre a *Pasteurella multocida* com (CMB - 5 mg/ml) e um efeito inibitório para o *Proteus mirabilis* (CMI – 20 mg/ml). *Ramaria botrytis* apresentou atividade contra *Enterococcus faecalis* e *L. monocytogenes* apresentando efeito bactericida para *a Pasteurella multocida* (CMB 20 mg/ml), *Streptococcus agalactiae* (CMB 20 mg/ml) e *Streptococcus pyogenes* (CMB 10 mg/ml). O extracto de *Leucopaxillus giganteus* inibiu o crescimento da *E. coli e Proteus mirabilis* sendo bactericida para a *Pasteurella multocida, Streptococcus pyogenes* e *Streptococcus agalactiae.*

Relativamente aos compostos puros testados (componentes principais dos extractos com melhor actividade) novamente sua acção foi corroborada principalmente sobre as bactérias Gram positivas. Verificamos que alguns compostos tinham maior atividade para MRSA que para MSSA.

Recorrendo a estudos de docking tentámos demonstrar que provavelmente os compostos 2,4-dihydroxybenzoic, protocatechuic, vanillic e p-coumaric inibem as PBP alteradas PBP2a.

Alguns dos extractos como a *Russula delica*, *Mycena rosea* e *Fistulina hepatica* apresentaram efeitos sinérgicos com antibióticos habitualmente usados na clínica para MRSA e *E. coli.*

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Tema da Tese: Characteristics of specific strains of *Listeria monocytogenes* potentiating persistence in food and food processing environments and relevance to food safety.

Listeriosis is a severe illness with a high mortality rate caused by *Listeria monocytogenes* after the ingestion of contaminated food with this bacterium. The ubiquitous nature and ability to grow in harsh conditions, makes this pathogen of special concern for the food industry, a known problem in Portugal. Despite worldwide efforts by researchers, food regulators and the food industry to reduce the incidence of listeriosis, this pathogen remains a critical threat to human health and the food supply.

The most important source of food contamination is cross-contamination or the transfer food processing environment (FPE) to foods.

Although FPE and equipment are regularly cleaned and sanitized, there are strains that recurrently occur in food (persistent strains) over several months or years while others are recovered only sporadically and the reason why is still not well understood. Also, some persistent strains were responsible for human cases of listeriosis.

Some authors have reported that the persistent strains appear to be better adapted to the FPE and possess specific characteristics that enhance survival to environmental stresses in the FPE, such as resistance to cleaning and sanitising agents.

Resistance of a subset collection of persistent and non-persistent isolates to disinfectants based on quaternary ammonium compounds, sodium hypochlorite, alcohol based, peracetic acid and hydrogen peroxide is being investigated. The susceptibility of the selected strains to these compounds is being assessed based upon survival to different exposure times (5 and 20 minutes) and different concentrations. Until now no differences in the resistance between persistent and non-persistent strains to the condition's tested has been observed.

Since 2003 a collaborative study with the main hospitals in Portugal and Escola Superior de Biotecnologia-UCP has been established which allowed to monitor the cases of listeriosis and PFGE characterizations of isolates. In January and February 2010 in Lisboa and Vale do Tejo region (LVTR) an increase in numbers of listeriosis was detected. The National Health and Food Authorities were alerted of this occurrence. To determine the possible food item responsible for the outbreak ESB collaborated with Direção Geral de Saúde, Administração Regional de Saúde (ARS) LVT and food authorities (ASAE). PFGE typing of food isolates revealed that the pulsotype responsible for the outbreak was isolated from samples of a kind of cheese produced in Alentejo.