Aluno: **Alexandra Sofia Moreira Amendolia da Costa Maia**

Orientador: **Professora Doutora Paula M. L. Castro e Professora Doutora Maria Elizabeth Tiritan**

Tema da Tese: **Fluoroquinolone antibiotics in environment: biodegradation studies and presence in several environmental matrices**

Sumário (máx. 3000 carateres, incluindo espaços)

Chiral drugs are amongst the most important groups of pharmaceuticals and its production, distribution, and use has been increasing over the years. Occurrence, fate and effects of pharmaceuticals in the environment is well reported but not much attention has been directed to enantioselective degradation processes regarding chiral compounds. Ofloxacin (OFL) and levofloxacin (LEV) are chiral fluoroquinolone antibiotics, extensively used worldwide. OFL is commercialized as a racemic mixture and LEV corresponds to the single (*S*)-enantiomer. The latter demonstrated to be up to 128 times more active against various bacteria, stating a higher antibacterial activity of the (*S*)-enantiomer compared to the (*R*)-isomer. Fluoroquinolones are considered persistent organic micropollutants, mostly due to its complex fluorinated structure that denotes their recalcitrant behavior.

The enantioselective degradation of OFL and LEV in a minimal salts medium was evaluated using an activated sludge inoculum (collected from a municipal wastewater treatment plant) and two different single bacterial strains *Labrys portucalensis* F11 and *Rhodococcus* sp. FP1, able to use several fluorinated organic compounds. In addition to the antibiotics, the assays were supplemented with a readily degradable carbon source, acetate at 5.9 mM. Studies were done under aerobic conditions, with constant shaking (150 rpm) at 25 °C during 46 and 28 days for activated sludge and isolated bacteria, respectively. The experiment was assembled protecting the flasks from light sources, in order to prevent photolytic degradation of the fluoroquinolones. An enantioselective LC-FD method using a ristocetin A-based chiral stationary phase was used to monitor the degradation studies.

Racemic-OFL was similarly consumed by the activated sludge inoculum and the isolated bacteria, exhibiting enantiomeric degradation extents between 51.8% (± 4.5%) and 66.8% (± 6.2%), for the (*R*)-enantiomer and the (*S*)-enantiomer, respectively. Non inoculated control assays showed no degradation of OFL enantiomers. In all the biotic conditions tested the (*S*)-enantiomer was slightly more consumed than the (*R*)-enantiomer. The chromatographic analysis of the degradation samples displayed the formation and accumulation of metabolites/degradation products during the biotic processes, proposing an incomplete mineralization of the analytes. In the assays with LEV the chromatographic data suggested the formation of the (*R*)-enantiomer along the degradation processes. The enantiomeric conversion was confirmed by LC-MS analysis of selected degradation samples. The exact mass profiles confirmed the presence of the (*R*)-enantiomer during the biodegradation in the assays initially supplemented with LEV ((*S*)-enantiomer), assuring that enantiomerization occurred during the biodegradation by the activated sludge inoculum and by strain F11.

Aluno: **Carlos André Narciso da Rocha**

Orientador: **Célia M. Manaia e José L. Martinez**

Tema da Tese: New insights into the environmental antibiotic resistome

Sumário (máx. 3000 carateres, incluindo espaços)

Rivers that cross urban areas, are normally inhabited by a mixture of strictly environmental bacteria and human impacted bacteria and therefore, may have a role on the spread of antibiotic resistance.[1]–[3] Besides the contamination with antibiotic resistant bacteria (ARB) and genes (ARG), it has been suggested that the chemical contamination with residues of disinfectants or antibiotics or heavy metals, also helps environmental bacterial to serve as a ARG reservoir for clinical pathogens.[1],[4]

A major aim of this thesis is to have new insights about the antibiotic resistance of human impacted habitats. With this objective in mind, two urban river transects were studied combining a comprehensive analysis of the bacterial diversity with the occurrence of multidrug resistance (MDR) phenotypes. For the identification of MDR bacteria, a combined approach based on culture-dependent methods and whole bacterial community analysis was used. MDR bacteria were observed to belong to the taxonomic groups predominating in the bacterial communities, i.e. the classes *Gamma-* and *Betaproteobacteria* and *Sphingobacteriia* and *Flavobacteriia* in particular of the genera *Pseudomonas*, *Acinetobacter* or *Stenotrophomonas*, known to be ubiquitous or *Chitinophaga* or *Chryseobacterium*, predominantly environmental. MDR bacteria of these and related groups are known to be widespread in water environments and have been, at least occasionally, associated with pathogenicity episodes. Although most of the resistance phenotypes were probably intrinsic in these species, these insights reinforce the relevance of the environment as potential supplier of bacteria and/or genetic determinants. Under favorable conditions, these resistance types may contribute to the spread of antibiotic resistance or act as clinically relevant opportunistic agents.

The fate of the ARB and ARG discharged into complex microbial communities is poorly understood. Some evidences suggest that they accumulate in the environment, as different studies demonstrate that old generation antibiotics, such as sulfamethoxazole or amoxicillin, present higher resistance prevalence than newer ones.[5]–[7] In order to study the fate of the ARB and ARG, assays were performed in microcosm settings composed of demineralized sterile water or wastewater samples of different origins, inoculated or not with known ARG harbored by known hosts. Variations of the load of the selected genes over time, suggested that the ARG *bla*$_{TEM}$ and *van*A has shorter persistence in water than the genes 16S rRNA or the class 1 integron integrase *int*I1 and that invasive ARG may tend to be eliminated by the autochthonous microbiota. The bacterial community composition shifts suggested that either the decrease of some populations (e.g. *Gammaproteobacteria*) or the increase of others (e.g. *Bacteroidia*) are associated with ARG elimination. These observations suggest the beneficial effect of the native microbial community on the elimination of invasive ARG.

References

[1] F. Baquero, J.-L. Martínez, and R. Cantón, “Antibiotics and antibiotic resistance in water environments,” *Curr. Opin. Biotechnol.*, vol. 19, no. 3, pp. 260–265, 2008.

[2] J. Tamames, J. J. Abellán, M. Pignatelli, A. Camacho, and A. Moya, “Environmental distribution of prokaryotic taxa.,” *BMC Microbiol.*, vol. 10, p. 85, 2010.

[3] I. Vaz-Moreira, O. C. Nunes, and C. M. Manaia, “Bacterial diversity and antibiotic resistance in water habitats: searching the links with the human microbiome,” *FEMS Microbiol. Rev.*, vol. 38, no. 4, pp. 761–778, 2014.

[4] T. U. Berendonk, C. M. Manaia, C. Merlin, D. Fatta-Kassinos, E. Cytryn, F. Walsh, H. Bürgmann, H. Sørum, M. Norström, and M.-N. Pons, “Tackling antibiotic resistance: the environmental framework,” *Nat. Rev. Microbiol.*, vol. 13, no. 5, pp. 310–317, 2015.

[5] A. Novo, S. André, P. Viana, O. C. Nunes, and C. M. Manaia, “Antibiotic resistance, antimicrobial residues and bacterial community composition in urban wastewater,” *Water Res.*, vol. 47, no. 5, pp. 1875–1887, 2013.

[6] L. Rizzo, C. Manaia, C. Merlin, T. Schwartz, C. Dagot, M. C. Ploy, I. Michael, and D. Fatta-Kassinos, “Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: a review,” *Sci. Total Environ.*, vol. 447, pp. 345–360, 2013.

[7] A. R. Varela, S. Andre, O. C. Nunes, and C. M. Manaia, “Insights into the relationship between antimicrobial residues and bacterial populations in a hospital-urban wastewater treatment plant system,” *Water Res.*, vol. 54, pp. 327–336, 2014.

Aluno: **Eduardo Manuel Aguiar da Costa**

Orientador: Prof. Manuela Pintado, Prof. Freni Tavaria, Eng. Jorge Faria

Tema da Tese: Chitosan in textiles: Towards a greener and functional solution

Sumário (máx. 3000 carateres, incluindo espaços)

Nowadays functional fabrics research is focused upon the integration of bioactive natural molecules into textiles thus creating active surfaces with anti-inflammatory or antimicrobial activity. However, these molecules must be previously validated before fabric integration and, in the particular case of antimicrobial activity, the rising levels of antibiotic resistance have led to the need of stricter and more thorough testing, mostly due to the lack of data regarding the effect of antimicrobials upon microbial biofilms.
Antibiotic resistance within biofilms is around 1000-fold higher than the values registered for planktonic growth. This is thought to be the underlying reason as to why antimicrobial treatments fail and it is estimated that *ca* 65-80% of all infections are biofilm related. Furthermore, antibiotic development pipelines rarely test the susceptibility of recalcitrant biofilm cells or utilize animal models in which bacteria form biofilm infections. In later years, chitosan, a polysaccharide with confirmed antimicrobial activity against planktonic cells, has gained a particular interest due to its biocompatibility and wide spectrum of activity, however little still is known regarding its activity against antibiotic resistant biofilms.
In order to assess chitosan's effect upon resistant microorganism's biofilms, four *Staphylococcus aureus* strains (two MRSA and two MSSA) were used in the present study and a two pronged approach was undertaken: first the planktonic MIC and MBC was determined for two chitosan molecular weights (MW) (624 kDa and 107 kDa). Next, the effect upon biofilms was assessed via determination of Minimal Biofilm Inhibition Concentrations (MBIC) and through inhibition of bacterial adhesion and mature biofilms.
The results showed that in the planktonic phase, chitosan was active at low concentrations, with both chitosans presenting an average MIC of 0.5 mg/ml. For biofilms, there was an increase in the chitosan concentration required to obtain an inhibitory activity, with values varying between 5 and 8 mg/ml. Despite this increase, chitosan presented high inhibition values for bacterial adhesion (50-90%) and was capable of reducing mature biofilms by 20% at least. Interestingly enough, throughout all the assays MRSA presented higher susceptibility to chitosan's activity.
In conclusion, chitosan displayed an evident strong effect against the tested *S. aureus* strains in planktonic and sessile state and MRSA biofilms were more sensitive to chitosan than MSSA. In all biofilm related assays both chitosans, exhibited high inhibition percentages preventing bacterial adhesion and disrupted mature biofilms. Furthermore, and contrary to traditional antimicrobials, chitosan's MBIC values only a 10-15 fold increase relatively to MIC value. Overall, these results show the potential of chitosan as a bioactive molecule for development of functional textiles.

Aluno: **FELIX POMPEYO FERRO MAYHUA**

Orientador: **Dra Celia Manaia / Dra. Ivone Vaz-Moreira**

Tema da Tese: **Ecology and antimicrobial resistance of *Ralstonia* spp. in the urban water cycle**

Sumário (máx. 3000 carateres, incluindo espaços)

Members of genus *Ralstonia* are ubiquitous in aquatic environments, including treated drinking water. The species *R. pickettii* and *R. mannitolilytica* are the most frequently detected. Members of these species can be opportunistic pathogens and display resistance phenotypes to a variety of antimicrobials. Based on a collection of strains of these species, were selected five isolates from hospital effluent, tap and bottled mineral water, which will be characterized aiming at the assessment of the potential selectors and/or environmental stressors on the development of antimicrobial resistance

Five strains, of four *R. pickettii* and one of *R. mannitolilytica* were selected. Four strains of *R. pickettii* isolated from hospital effluent, mineral and tap water, and one of *R. mannitolilytica* from tap water were selected for this study. Strains were identified based on the 16S rRNA gene sequence analysis, and characterized for their antimicrobial susceptibility phenotypes using the disk diffusion and/or the microdilution method to determine the minimal inhibitory concentrations (MICs). Growth kinetic parameters and capacity to form biofilm were determined in the absence and in the presence of the antimicrobial agents gentamicin or arsenite, at concentrations below or close to the MIC values.

Gentamicin resistance, observed in three of the *R. pickettii* strains (one from each type of water), coincided with the highest arsenite MIC values (>256 mg/L and =1.4 mM). In contrast, in gentamicin susceptible strains (MIC value of <6mg/L) the arsenite MIC values was of 0.05 mM. In Mueller-Hinton broth at 30 ºC, the growth rates of these strains ranged 0.38-0.43 h^{-1} in stressor-free medium and 0.13-0.40 h^{-1} or 0.28-0.41 h^{-1} in the presence of gentamicin or arsenite, in concentrations close to the respective MIC value. Arsenite and gentamicin affected the length of the lag phase and growth yield. In gentamicin resistant strains, the presence of gentamicin caused a significant reduction of growth rate and the presence the arsenite led to significant increase of the lag phase

In gentamicin resistant strains, the presence of gentamicin and streptomycin, in comparison with antimicrobial-free culture medium, was observed to significantly increase the formation of biofilm. This effect was not observed in the presence of arsenite.

Based on the hypothesis that gentamicin resistant strains could be significantly more resistant to UV, the inactivation rate of the five strains after exposure to germicide radiation was compared. No significant differences were observed among the five strains.

To assess the ability of *R. pickettii* to acquire new resistance phenotypes in the presence of stressors, two strains, one resistant and one susceptible to gentamicin, were successively transferred in the presence of increasing concentrations of gentamicin or to increasing exposure times of UV. The strains artificially evolved in presence of gentamicin or UV radiation suffered slight modifications in the antibiotic resistance profile. Putative associated genetic variations will be surveyed in the future.

Further studies, including comparative genomics approaches ongoing in our group, will seek to find genetic elements that may explain some of the above reported observations. For example, the genetic determinants associated with integrative conjugative elements were observed only in gentamicin-resistant and arsenite-tolerant isolates.

Aluno: **Fernanda Rosa Assis**

Orientador: **Alcina Maria Miranda Bernardo**

Co-orientador: Rui Manuel Santos Costa de Morais

Tema da Tese: **Modelling and optimization of osmotic dehydration combined with other methods for drying fruits**

Sumário (máx. 3000 carateres, incluindo espaços)

The development of new manufacturing processes has become necessary for the maintenance of the nutritional properties and quality, as well as the sensory characteristics of products processed from fruits. The dehydration confers properties, such as stability at room temperature, convenience in transportation and product versatility. This process consists of reducing the water content of the food, decreasing the water activity (a_w), thereby inhibiting the growth of microorganisms and delaying the deterioration of physico-chemical origin. The osmotic dehydration (OD) is a method to partially reduce the water content of food, aiming to increase its shelf life or as a pre-treatment in the dehydration processing of foods. The OD combined with other type of drying, such as convective, vacuum, microwave and freeze-drying, applied to fruits, vegetables, fish and meat has been proposed by many researchers. The use of sorbitol as osmotic agent in OD of fruits is an alternative to replace the sucrose because of its low calories and relative sweetness of around 60% compared to sucrose. Mathematical models have been proposed with the objective to predict the mass transfer kinetics of the OD process, and they may be classified as empirical and semi-empirical, mechanistic and phenomenological.

The aim of this work was 1) to perform a comparative study on the adequacy of some models to fit experimental data of apple cubes (variety Royal Gala) osmotically dehydrated at different temperatures — 25, 40 and 60 ºC —, using two solutes — sucrose and sorbitol — and two mass ratios of sample to solution — 1:4 and 1:10; 2) to carry out the OD of apple cubes at 60 ºC for 8 h, using sucrose or sorbitol at the mass ratio sample/ solution of 1:4, followed by hot air drying, and to study the effect of the air temperature — 25, 55, 70 and 80 ºC — on the mass transfer kinetics of the air drying and on the water activity of the product. Azuara's, Peleg's, Page's and Weibull's models could fit the experimental data of OD — WL and SG —, but the Penetration model resulted in a worse fit. Crank's model presented good correlations only for WL, and the water effective diffusivity was between 8.85×10^{-11} and 1.38×10^{-9} $m^2.s^{-1}$. The increase of the temperature and the use of sorbitol as osmotic agent resulted in an increase of the process rate, but the mass ratio of sample to solution did not affect the mass transfer kinetics. In the hot air drying, the OD pre-treatment and increase of the air temperature resulted in an increased water loss rate and reduction of a_w during the air drying of apple cubes. The osmotic agent used in the OD was not relevant to the kinetics, but the use of sorbitol solution produced dried samples with lower a_w.

Aluno: **Giovânia Carvalho Araújo**

Orientador: **Paula Castro**

Co-supervisor**: Nadine Sousa**

Tema da Tese: **Symbionts as trees growth promoters under stress conditions**

Sumário (máx. 3000 carateres, incluindo espaços)

The objective of this programme is the selection of plant growth-promoting bacteria (PGPR) and ectomycorrhizal fungi (ECMF) able to establish symbiosis with *Q. suber* and *P. pinea*, to promote better growth and survival in the field after transplant. Initially, the inoculation of *Quercus suber* L. seedlings was tested with two bio-inoculants, a commercial product containing *Pisolithus tinctorius*, *Scleroderma* sp. and six bacterial species and a non-commercial bacterial and fungal dual inoculum (*Suillus granulatus* + *Mesohrizobium* sp). The experiment was conducted in different forest nurseries located in two Portuguese regions, Amarante and Buçaco. To identify the predominant ECMF species present on both nurseries, analysis of molecular cloning of relevant samples were performed. The ectomycorrhizal community was assessed by denaturing gradient gel electrophoresis. In both nurseries, the dual inoculum was the most efficient in promoting shoot development and improving Nitrogen use efficiency. *S. granulatus* and *P. tinctorius* persisted in the root system after six months whereas *Scleroderma* sp. was not detected. For a deeper insight into the signaling involved during mycorrhizal establishment, with and without the presence of bacteria, the role of flavonoids is currently being investigated. For this, preliminary tests were conducted to select the appropriate fungus-bacteria combination. The selected fungi were *Lactarius delicious*, *P. tinctorius* and two isolates of *S. granulatus*. The bacteria chosen was *Bacillus subtilis* as it proved to significantly affect fungal growth (either by promoting or inhibiting) and additionally due to its potential PGPR skills (Pereira & Castro, 2014). An *in vitro* study was set up to evaluate the process of of mycorrhization, determining the metabolites resulting from contact of fungi with the plant root and changes in plant tissues. Sample treatment for the quantification of flavonoids by HPLC has been optimized. Mycorrhization systems are now being established on square petri dish containing autoclaved substrate. Germinated *Pinus pinea* seedlings will be placed in the substrate and inoculated with fungi and bacteria, for a total of 7 inoculation treatments plus the control. The systems will be maintained in a controlled growth room in a time course experiment where a destructive sample will be performed after 1, 7, 15, 30 and 60 days. Five replicates per treatment will be analyzed. Analysis of biometric parameters (height, root length, diameter, and weight), nutritional status and metabolites in the plant and the rhizosphere through analysis of HPLC will be performed. At the end of the experiment, mycorrhized seedlings will be transplanted to different substrates to evaluate their performance.

Aluno: Glenise Bierhalz Voss

Orientador: Maria Manuela Estevez Pintado e Luísa Maria Valente

Tema da Tese: Production and characterization of bioactive peptide extracts from okara (byproduct of soy milk) with potential application in food and feed

Sumário (máx. 3000 carateres, incluindo espaços)

Soybeans are the main oilseed produced and consumed worldwide and it has great commercial interest, especially, because of the oil extraction, soy proteins and soymilk. Soymilk is achieved by aqueous extraction of whole soybeans. During this process, a byproduct known as okara rich in fiber, protein and fat, is obtained. Okara is produced in high amounts, since for each 1 kg of processed soybeans about 1.1 kg of okara is produced. This byproduct has a high nutritive value, as previously mentioned and studies reported that protein can be produced from the okara and that the protein isolates are characterized as having good amino acid profile and showing good digestibility.

Therefore, the general objective of this study was to evaluate the chemical composition (protein, fiber, lipids and ash), microbiological stability, antioxidant capacity and isoflavones in fresh okara stored at 4 ºC and dried okara (previously autoclaved - OA and not autoclaved - ONA) stored at room temperature for 60 day. Additionally, another objective of this study was to evaluate the enzymatic hydrolysis in dry okara (OA and ONA) with an enzyme extract from *Cynara cardunculus* and alcalase, using two different hydrolysis parameters (reaction time and ratio of enzyme/substrate) and characterize its biological activities (antioxidant and antihypertensive activities) and free amino acids.

With this study it was observed that okara showed a rich nutritional composition − ca. 52% of fiber, 30% of protein and 4-11% of lipids. The okara's lipid profile showed high and valuable level of PUFA and MUFA, but presented significant differences between treatments. Nevertheless, the dry ONA showed ten times more acidity than the dry OA. The fresh okara showed the highest antioxidant activity and total phenols.

In relation the antioxidant activity in enzymatic hydrolysis with alcalase and *Cynara cardunculus* extract, an increase was observed for both samples for longer hydrolysis time and higher concentrations of E/S, however for OA this value was higher than for ONA. Besides, for the antihypertensive activity, under all experimental conditions, OA hydrolysates showed IC50 values lower than the ONA hydrolysates, thus indicating higher ACE-inhibitory activity, furthermore the hydrolysates showed higher antihypertensive activity than that found in previous studies for soy peptide extracts. Concerning free-amino acid profiles both hydrolyzed samples showed good profile in terms of essential amino acids, but the hydrolysates produced with *Cynara cardunculus* extract showed higher concentration of essential amino acid than in hydrolysates by alcalase. It can hence be concluded that the peptide extracts obtained from enzymatic hydrolysis of okara protein with both enzymes show high nutritional and bioactive potential that was generally enhanced after the pre-sterilization treatment. Furthermore, the nutritional profile and bioactivity of this byproduct suggests okara as a valuable ingredient to be used in the development of functional foods.

Aluno: **Joana Odila Mendes Sá Pereira**

Orientador: **Doutora Manuela Pintado, Doutora Ana Gomes**

Tema da Tese: **Development and characterization of bioactive edible films and coatings by incorporation of functional bacteria**

Sumário (máx. 3000 carateres, incluindo espaços)

Innovations are constantly appearing in food packaging, enabling the development of new foods that may offer a more efficient quality preservation system, reduced environmental impact and no negative impact on the consumer. Edible coatings and films are, among these innovations, a good attempt to increase the storability of foods by controlling gas exchange, moisture, pathogens' growth and oxidative reaction rates. The use of edible coatings and films formulated with bioactive compounds in food products opens new possibilities for these to act as carriers for functional lactic acid bacteria. In this case, bioactive coatings/films can be obtained by microorganism's immobilization in the film, which, when in contact with the food, may release relevant bioactive principles and promote competition *in situ*, and can be complemented with health benefits.

The aim of this study is the incorporation of functional microorganisms in edible protein coatings/films their characterization and their application for coating food products toward higher quality and safety.

The results achieved up to the present moment, reveal that it is possible to incorporate efficiently viable probiotic strains in the films, maintain their stability throughout storage and maintain physico-chemical characteristics of films.

Results from incorporation of *Bifidobacterium animalis* BB-12 and *Lactobacillus casei*-01 demonstrated a viability loss of ca. 3 log cycles (reaching 10^6 CFU/g film) until 60 d at both 23 and 4 ºC. *Bifidobacterium animalis* BB-12 remained viable at higher level (10^8 CFU/g film) during the study.

Physical properties, namely color, water activity, thickness, and texture characteristics were maintained stable throughout storage at both temperatures.

Furthermore, it was possible to develop and evaluate the antimicrobial efficiency of edible coatings incorporated with probiotics on sliced ham preservation.

The physicochemical analyses showed that application of coating decreased water and weight loss on the ham surface throughout storage when compared to the uncoated ham, independently of the incorporated strain; demonstrates that the edible coatings could be used as a suitable alternative to extend shelf-life. Moreover, color analysis showed that sliced ham immersed in the film solution, didn´t exhibit a color change, comparatively to uncoated slices.

In terms of microbial response, the edible coatings successfully inhibited detectable growth ($<10^2$ CFU g^{-1}) of *Staphylococcus* spp., *Pseudomonas* spp., *Enterobacteriaceae*, and yeasts and molds – thus demonstrating ability to guarantee protection of ham against spoilage and pathogenic microorganisms

growth for, at least, 35 days of storage. Furthermore, probiotic bacteria were not inhibited and their numbers were maintained at high and constant levels of ca. 10^6 CFU/g throughout storage, enabling the slice of ham to act as a suitable carrier for the beneficial bacteria providing the associated positive effect to the consumers.

Aluno: **Maria Margarida Lobo Machado Sousa Nazareth Almeida Sampaio**

Orientador/Co-orientador: **Prof. Doutora Carla Rêgo /Prof. Doutora Elisabete Pinto**

Tema da Tese: **Padrão alimentar precoce, estado nutricional e crescimento em crianças portuguesas com 12-36 meses: um estudo representativo nacional.**

Sumário (máx. 3000 carateres, incluindo espaços)

O EPACI Portugal2012 (Estudo do Padrão Alimentar e de Crescimento na Infância) pretende avaliar o padrão de crescimento, o estado de nutrição e os hábitos alimentares de crianças dos 12 aos 36 meses de idade, residentes em Portugal Continental.

Um dos grandes objectivos deste projecto consiste na avaliação da ingestão alimentar das crianças portuguesas. A dificuldade na identificação das melhores recomendações a utilizar na avaliação da ingestão alimentar das crianças portuguesas, justificou a elaboração de uma revisão desta temática e cujo resumo apresento abaixo.

Introdução: A alimentação diária deve suprir as necessidades nutricionais e a sua adequação é fundamental para um crescimento e desenvolvimento saudáveis ao longo da infância e da adolescência. Não existem recomendações nutricionais portuguesas e na ausência destas, não há um consenso relativamente às recomendações que deverão ser utilizadas em Portugal. Objectivos: Sistematizar e comparar as recomendações nutricionais na infância e na adolescência (0-18 anos) e contribuir para a adopção de recomendações a utilizar para a população pediátrica portuguesa. Métodos: Seleccionaram-se as recomendações mais utilizadas para crianças e adolescentes, tendo por base uma revisão das publicações na base PubMed® nos últimos 10 anos: as do FNB/IOM (EUA/Canadá), as da FAO/OMS (Mundiais) e as da EFSA/CE (Europeias). Posteriormente, analisaram-se todos os documentos existentes relativos a estas recomendações nutricionais. Resultados: Os três Comités considerados apresentam critérios diferentes, nomeadamente na estratificação por idade que fazem, para apresentar as recomendações e na terminologia utilizada. As recomendações da EFSA/CE destinam-se à população europeia e têm por base uma metodologia sólida, incluindo recomendações dos outros dois Comités analisados, sendo também as mais recentes, no entanto as recomendações da FNB/IOM são as mais utilizadas. Os valores recomendados para energia, proteína e lípidos não apresentam grandes variações entre Comités. Relativamente aos hidratos de carbono, as recomendações da FAO/OMS são as mais elevadas. No que diz respeito às vitaminas e minerais, de uma forma geral, as recomendações para a vitamina B1, ácido pantoténico, cálcio, selénio, zinco e iodo são semelhantes para os três Comités, apresentando algumas variações para as restantes vitaminas e minerais. Conclusões: A adopção oficial de recomendações nutricionais para a população portuguesa é importante e urgente, para permitir a uniformização de critérios e comparar resultados. A solidez metodológica e a actualidade das recomendações da EFSA/CE levam os autores a considerá-las uma opção a recomendar.

Este artigo de revisão foi submetido a uma revista nacional e encontra-se em fase de avaliação.

Neste momento, e por forma a dar reposta aos restantes objectivos delineados, tenho vindo a trabalhar em dois artigos relacionados com a caracterização do estado nutricional das crianças portuguesas (0-36 meses).

Aluno: **Sara Nunes da Costa e Silva**

Orientador: **Prof. Manuela Pintado, Prof. Rui Morais, Prof. Conceição Calhau**

Tema da Tese: Study of Vaccinium corymbosum berries and leaves toward incorporation in functional foods

Sumário (máx. 3000 carateres, incluindo espaços)

Blueberries, the fruits of *Vaccinium corymbosum,* are among the fruits with the highest anthocyanin contents, while their leaves are reported as rich in phenolics acids. So it is important to understand the role and potential of both anthocyanins and phenolic acids, as antimicrobial agents. Leaf phenolic acids have been previously found to possess an interesting antibiofilm activity and optimization of the extraction process was undertaken.

For blueberry extract, after the definition of the extraction protocol, an audit was undertaken in order to better understand the limitations of the extraction process. Afterwards the purified extract's capacity to inhibit the growth and adhesion of several pathogens, some of which were multiresistant bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, *Acinetobacter baumannii*, *Proteus mirabilis* and a methicillin sensitive and a methicillin resistant *Staphylococcus aureus*) was determined. Furthermore, in an attempt to determine if the anthocyanins were responsible for some antimicrobial activity observed, the antimicrobial activity of pure malvidin-3-glucoside (the main anthocyanin present in the extract) was determined.

On another note, as previous studies had shown that blueberry extracts were unable to reduce the growth of lactic and probiotic bacteria, the extract's effect upon five different probiotic bacteria (*Lactobacillus acidophilus* Ki, *Lactobacillus rhamnosus* R11*, Lactobacillus plantarum* 299v, *Bifidobecterium animalis* Bo and *Bifidobacterium animalis* Bb12) was also studied. Potential food pathogens and probiotic bacteria were exposed to extract (1 mg/mL) and bacterial growth was monitored as well as the production of organic acids by the probiotics. Furthermore, the extract's effect upon probiotic and pathogen adhesion, in single species and multispecies cultures, was also evaluated.

Additionally, as blueberries are described as possessing a good antioxidant capacity an attempt is being made in order to improve a traditional DNA/agarose gel electrophoresis pre-existing method. The traditional approach has a major limitation; results are typically reported qualitatively either there is a band or not. We are developing an improved method in order to use the band intensity and the distance to the well to determine the overall degradation of DNA.

Aluno: **Tânia Cristina Ferreira Ribas Vaz Pedro**

Orientador: **António Osmaro Santos Silva Rangel, Ildikó Vargáné Tóth**

Tema da Tese: **Flow-based methods for monitoring environmental samples**

Sumário (máx. 3000 carateres, incluindo espaços)

Spectrophotometric assays play an important role in everyday laboratory practice. High throughput assays became indispensable in real life laboratory of any field of interest such as environment, biochemistry, biotechnology or food quality. The first year of the PhD was devoted to the development of miniaturized techniques for the preliminary studies involving metals and complexants. So, spectrophotometric titration assays, Jobs method of continuous variation and calibration curve methods were adapted to the microplate format. The developed protocols were based on the study of the reaction of different metal with two different complexing agents.

The second year of the PhD was devoted to the development of a flow injection methodology with solid phase extraction and spectrophotometric detection for the determination of total content of zinc in plant digests.

Zinc plays an important role in plant metabolism; being the most significant its activity as component of various enzymes. However, it is very toxic at high concentrations. Its concentration is related with the chemical composition of the growth media [1]. Zinc is widely used in many industries and this way it is introduced in the environment.

Several methods are available for zinc determination in plants digests, such as Atomic Absorption Spectrometry (AAS) or Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). These methods present high selectivity and low limits of detection. However, those equipments present relatively high cost and uses toxic gases [2].

The developed method is based on a solid phase extraction for zinc pre-concentration and removal of some interferences, and the colorimetric determination involving Zincon. To implement this approach, an injector commutator and a multi-reflection flow cell were used.

The developed system provides a simple and reliable determination of zinc in plants. When applied to plants digests, the results were in agreement with those obtained with reference procedure (AAS).

The next step in the PhD programme is to develop a methodology for the fluorescent determination of primary amines in natural waters, using fluorescamine as derivatisation reagent.

[1] Kabata-Pendias A. *Trace Elements in Soils and Plants (4th ed.)* **2011**. CRC Press, Boca Raton, USA

[2] Kalra Y.P. *Handbook of Reference Methods for Plant Analysis* **1998**. CRC Press, Boca Raton, USA

Acknowledgements: T.C.F. Ribas thanks to Fundação para a Ciência e a Tecnologia (FCT, Portugal) and Fundo Social Europeu for the Grant SFRH/BD/91820/2012. This work was also supported by National Funds from FCT – Fundação para a Ciência e a Tecnologia through the projects PTDC/AAG-MAA/5887/2014 and UID/Multi/50016/2013.

Aluno: **Vânia Sofia de Sousa Bessa**

Orientador: **Doutora Paula M. L. Castro**

Co-Orientador**: Doutora Irina Sousa Moreira**

Tema da Tese: **Biodegradation of fluorinated pharmaceuticals – enrichment of degrading microorganisms and study of biotreatment systems**

Sumário (máx. 3000 carateres, incluindo espaços)

Pharmaceutical compounds have received increasing attention as emerging organic pollutants due to their frequent occurrence in the environment and potential adverse effects on ecosystems and to human health. Their presence in wastewater is a matter of great concern. As current wastewater treatment plants (WWTPs) are not designed to remove these emerging pollutants, excreted pharmaceutical metabolites and some unchanged forms of these compounds enter the ecosystem.
Carbamazepine (antiepileptic) and diclofenac (nonsteroidal anti-inflammatory) have been pointed out as important markers for pharmaceuticals' environmental pollution, as they are frequently detected in water bodies, due to their high removal persistence in WWTPs. Moreover, field data indicate that these compounds are environmentally persistent. Microorganisms are well-known key players in degrading pollutants in the ecosystems through metabolic and/or co-metabolic pathways. Many authors have deemed that this is one of the most important processes for eliminating the majority of xenobiotics, including pharmaceuticals. Knowledge on microbial processes for biodegradation of CBZ and DCF is scarce.
In the present work the enrichment, isolation and identification of bacterial strains able to degrade CBZ and DCF retrieved from a domestic activated sludge treatment plant is described. Also, the biodegradation potential of a bacterial strain isolated in our laboratory – *Labrys portucalensis* F11 – was evaluated. Degradation of these compounds as single carbon source and in co-metabolism was evaluated. From the selective enrichments, one strain able to degrade DCF and two strains able to degrade CBZ were isolated. The strains were identified by 16S rRNA gene sequencing. Strain *Brevibacterium* D4 was able to biodegrade 35% of 0.03 mM of DCF as a sole carbon source; periodic feeding with acetate (Ac) as a supplementary carbon source resulted in enhancing biodegradation to levels up to 90%, with a concomitant increase in biodegradation rate. Strains *Starkeya* sp. C11 and *Rhizobium* sp. C12 were able to biodegrade 30% of 0.04 mM of CBZ as a sole carbon source; supplementation with Ac did not improve the biodegradation of carbamazepine by these strains. Regarding strain F11, 25% of 0.03 mM of DCF as a sole carbon source was biodegraded; when supplemented with Ac, a 61% of biodegradation was achieved; periodic feeding with Ac resulted in 100% of biodegradation. Strain F11 was able to biodegrade 27% of 0.04 mM of CBZ as a sole carbon source; the supplementation with Ac increased CBZ biodegradation to 47%; periodic feeding with Ac resulted in enhancing biodegradation to levels up to 75%.

These bacterial strains may help elucidating the mechanisms of biodegradation of these compounds being potentially useful for bioaugmentation strategies.