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

Sumário (máx. 3000 carateres, incluindo espaços)

Acinetobacter spp. are ubiquitously distributed in nature. In the past, these microorganisms were considered saprophytes of little clinical importance. However 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 aim of this study is to identify and characterize *Acinetobacter* spp. present in food, specifically: assess the most prevalent species; genotypic characterization of isolates from different origins; and evaluation of resistance to antibiotics.

The presence of *Acinetobacter* spp. was investigated in 50 samples of meat (pork, turkey, chicken and cow). The organism was found in all the samples; a total of 223 isolates were recovered and subtyped by PFGE (*Pulsed Field Gel Electrophoresis*). One hundred and seventy different PFGE types were observed; one isolate of each PFGE type was selected for identification to species level by *rpoB* gene analysis. Isolates were distributed among 13 species; the predominant species recovered were *A. guillouiae* (18%), *A. johnsonii* (13.3%) and *A. bereziniae* (13.3%). Other species, of clinical significance, were also found such as *A. pittii* (9.4%), *A. seifertii* (6.3%), *A. baumannii* (4.7%) and *A. nosocomialis*

(3.1%). Eight strains may represent distinct (novel) species since their similarity value of *rpoB* sequence was lower than 95 % with type strains of *Acinetobacter* species.

Antibiotic resistance phenotypes were determined using disk diffusion and agar dilution methods, according to standard recommendations (Clinical and Laboratory Standards Institute, 2012). The highest resistances were observed for piperacillin (69.5%), piperacillin-tazobactam (66.5%), ceftazidime (44.7%), ciprofloxacin (44.1%) and colistin (41.8%).

This study demonstrated that meat may be a reservoir of *Acinetobacter* spp., including those forming the Abc complex.

Aluno: Bárbara Filipa Santos Ramos

Orientador: Cristina L.M. Silva, Paula Teixeira e Teresa Brandão

Tema da Tese: Biological Control of *Listeria monocytogenes* in fresh vegetables

Sumário (máx. 3000 carateres, incluindo espaços)

Raw vegetables have been identified as a vehicle of transmission of foodborne outbreaks and play an important role in listeriosis epidemiology. This is of special concern because this kind of food are eaten raw and rely only on cold storage to maintain their safety, however *Listeria* has the ability to survive and multiply at refrigeration temperatures.

Consumers' concern about chemical residues in food products has led the food industry to seek novel and alternative technologies to improve the quality and safety of fresh and minimally processed vegetables. Lactic acid bacteria (LAB) strains naturally present in vegetables are good candidates for improving the microbiological safety of minimally processed produce.

Pediococcus pentosaceus DT016, a bacteriocin producing strain, was isolated from fresh lettuce. The bacteriocin, named pediocin DT016, exhibits activity against *L. monocytogenes*, *L. innocu*a and *E. faecalis*, is stable to a wide range of pH values and maintains the antibacterial activity at refrigeration temperature (4 ºC).

The ability of *P. pentosaceus* to supress *Listeria. monocytogenes* under refrigerated storage was assessed in fresh vegetables. The survival of the pathogen was evaluated along time in fresh lettuce, rocket salad, parsley and spinach in the presence or absence of the protective culture. The vegetables were inoculated with a *L. monocytogenes* cocktail at levels of about 6 and 4 log CFU/g and stored at 4 ºC. The pathogen load in the vegetables with the protective culture decreases along storage. Contrary to that, in the fresh vegetables *L. monocytogenes* was able to grow and an increasing load was observed along refrigerated storage. At the last day of storage, the presence of the protective culture resulted in a minimum pathogen reduction of 1.4 log CFU/g when compared with the pathogen load in fresh vegetables. *P. pentosaceus* DT016 showed to be a promising alternative to maintain the safety of fresh vegetables kept under cold storage.

Additionally, in another experiment, it was determined whether it would be possible to use pediocin DT016 as a biopreservation factor in a washing step for fresh or minimally processed vegetables. Fresh lettuce, rocket salad, parsley and spinach were contaminated with *L. monocytogenes* and the pathogen load was studied after washing with: water, a commercial sodium hypochlorite solution (AMUKINA) and the pediocin solution, along storage at 4 ºC. The pediocin solution immediately after washing significantly reduced the initial *L. monocytogenes* load and along storage inhibited the pathogen proliferation. At the end of storage, the pathogen load in the vegetables washed with the pediocin was lower than in the

vegetables washed with water and AMUKINA, by a minimum of 3.2 and 2.7 log CFU/g, respectively. Therefore, the application of washing solutions containing pediocin DT016 is a very promising method to reduce and inhibit *L. monocytogenes* proliferation in fresh vegetables at refrigerated storage.

Aluno: Carla Sofia Sancho dos Santos

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

Sumário (máx. 3000 carateres, incluindo espaços)

Iron deficiency chlorosis (IDC) is a serious physiological disorder, prevalent on calcareous soil, contributing to decreased crop growth and yield. Its main visible symptom is leaf yellowing due to decreased chlorophyll concentrations. Understanding the mechanisms underlying iron uptake, trafficking and homeostasis is essential in order to prevent IDC and enhance plant productivity. Although Fe is an important element of enzymes involved in the tetrapyrrole and antioxidative system, studies on the impact of Fe deficiency on the regulation of these metabolic systems are still scarce. The tetrapyrrole cycle is involved in the biosynthesis of chlorophyll and heme, and it starts with the formation of 5-aminolevulinic acid (ALA). As iron is necessary for hemoproteins (heme containing proteins, such as Fe reductases, catalases and peroxidases) and chlorophyll synthesis, its absence leads to decreased photosynthetic capacity and damaged antioxidative defence. In the current study, soybean plants were grown hydroponically under Fe sufficient (20 µM) and Fe deficient (0 µM) conditions for 14 days and photosynthetic pigments, Fe accumulation, root reductase activity, ALA and hemin (the oxidized version of heme) concentration and antioxidant enzymes activity was evaluated. Under Fe limitation, shoots had a significant decrease of anthocyanins (37%), and a 30% decrease for chlorophyll a, chlorophyll b, total chlorophylls and carotenoids. A significant decrease in Fe accumulation in both roots (21%) and shoots (81%) was also observed ($P < 0.05$). Fe deficiency had a strong impact on ALA and hemin tissue accumulation: in the roots there was a reduction of both metabolites (29% for ALA and 42% for hemin), whereas in the shoots only hemin was affected (51% reduction) ($P < 0.05$). With regards to the antioxidative system, the activity of catalase and ascorbate peroxidase (heme-group containing enzymes) decreased proportionally to the decrease in hemin concentration under Fe-deficiency, showing a relation between heme accumulation and antioxidative enzyme activity. Root ferric chelate reductase activity and shoot glutathione reductase activity were increased by 79 and 45%, respectively. This study reveals the impact of Fe deficiency on crucial components of the tetrapyrrole and antioxidative systems, and further elucidates the biochemical responses of soybean to Fe deprivation.

Aluno: Maria Filomena de Jesus Raposo

Orientador: Rui Manuel S. C. de Morais e Alcina Maria M. Bernardo de Morais

Tema da Tese: Biotechnology of microalgae: influence of the biochemical parameters and culture conditions on the production of biomass and bioactive compounds

Sumário (máx. 3000 carateres, incluindo espaços)

Microalgae are rich sources of many bioactive compounds, such as phytosterols, proteins (including antioxidant enzymes), polyunsaturated fatty acids (including ω-3 and ω-6 FA), carotenoids and other pigments. Carotenoids, for example, are a group of compounds with strong antioxidant activity, which may have applications as therapeutics to prevent/treat a wide variety of diseases. Additionally, there is a wide variety of microalgae producing different polysaccharides. Most of the times these polymers are incorporated into the algal biomass, but some marine microalgae secrete the excess of the polysaccharides out into the culture medium. There is a body of evidence on the various applications of those polymers, including in human health.

One of the objectives of this PhD program is to study the influence of several factors, such as light, salinity or the enrichment of the culture medium, on the production of microalgal biomass and their bioactive compounds. The quality and applications of those compounds are also focused.

The first task was already carried out, and the knowledge on the compounds produced by microalgae was put up-to-date. For the accomplishment of the next tasks, some algal species were grown, under various conditions, namely by varying the salinity for *Dunaliella salina,* by adding different plant growth regulators to the culture medium of *Haematoccus pluvialis* and *D. salina,* and by varying the magnesium and sulphate content in the growth medium of *Porphyridium cruentum*. The addition of plant hormones to the culture medium effectively enhanced growth of microalgae, especially *D. salina*. In fact, when under a high salinity, the algal biomass reached an increase of up to 410%; *H. pluvialis* also showed a significant increase in the growth rate when the ratio auxin to cytokinin was 1 (equal concentrations of 1.0 mg l^{-1} of both growth regulators) with 290% more cells than the control. Additionally, the implementation of the culture medium with magnesium (as $MgCl_2$) strongly influenced the growth of *P. cruentum*. Moreover, an increase of the sulphate content in the culture medium (as $MgSO_4$) induced an increase in the protein levels of the biomass from *P. cruentum* and in the sulphate residues of its EPS, which presented a strong activity against viruses.

Aluno: Maria Manuela Faria Amorim

Orientador: Maria Manuela Estevez Pintado

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

Sumário (máx. 3000 carateres, incluindo espaços)

Spent brewer's yeast (*Saccharomyces cerevisiae*) is a by-product of the brewing process and represents 2–3% of total beer production. Most of this yeast is sold as animal feed at very low price or has to be disposed as waste. However, this by-product contains certain valuable cellular components, e.g. structural cell wall polymer β-glucans and proteins. These compounds, besides its nutritional value, may present several bioactive properties - antihipertensive, antioxidant, immunostimulant, and prebiotic activities, among others. However, only few studies have screened these functionalities. Thus, the general objective of this work was to use spent brewer's yeast to obtain new functional ingredients (in particularly peptide concentrates and β-glucans), and characterize its composition and biological activities, to finally prove its potential in the development of new functional foods.

In order to obtain peptide and polysaccharide enriched extracts, the spent brewer´s yeast (provided by Unicer) was initially submitted to autolysis. Afterwards, the mixture was concentrated by ultrafiltration with a 10 kDa cut-off membrane and hydrolyzed with proteases from aqueous extract of *Cynara cardunculus*. Retentate and filtrate were nanofiltrated with 3 kDa cut-off membrane, and resulting extracts concentrated by reverse osmosis and dried by freeze drying. Each yeast peptide extract obtained were tested concerning antihypertensive activity by *in vitro* Angiotensin-converting enzyme inhibition test, where all fractions showed promising result values, mainly high molecular weight filtrate <3kDa fraction with IC50 less than 100 µg/mL. The *in vivo* effect of those extract was studied using spontaneously hypertensive rats (SHR) and metabolic syndrome where rats were exposed to a high-fat and standard diet (with or without peptide extracts) for 5 weeks and several key parameters were monitored, namely blood pressure, glucose and insulin tolerance; adipose tissue morphology; weight control, etc. Results showed a significant arterial pressure decrease, especially systolic, combined with a positive effect on weight control, glycemic index and energy gain.

The potential anti-ulcerative *in vivo* effect of those bioactive factions was also studied and results showed a protective effect on gastric mucosa against ulcerative lesions caused by ethanol. On the other hand, the screening of antiproliferative effect on different types of cancer cells lines was also investigated. Extracts showed cytotoxic effect against leukemia cells. All these results are attracting attention in the search of

new ingredients used in the prevention and control of these diseases. Therefore the results presented in this study, highlight yeast peptide extracts, in particular <3kDa as promising ingredient for further utilization in special foods areas and dietary supplements that can be used in the prevention of chronic diseases. Such evidence is being reported for the first time for this kind of extracts.