Student: Adriana Nunes de Lima

Supervisor: Jose Antonio G. Couto

Co-supervisor: Francisco M. M. S.Campos

Thesis Tittle: Production of volatile phenols in wines by Brettanomyces/Dekkera: The influence of the grape variety.

Abstract

The volatile phenols production is of great concern to the wine industry, due the fact of being responsible for unpleasant aromas that affect the wine quality. It's a global problem and there are many lines of research concerning this matter. The ethylphenols, resulting from the degradation of hydroxycinnamic acids due to microbial activity (mainly the yeast Brettanomyces/Dekkera) give the wine an aroma of "horse sweat", "leather" and "clove" especially at relatively high concentrations. The control of the volatile phenols formation is one of the most important microbiological challenges in the modern wine production, since it is responsible for significant economic losses throughout the world. The aim of this project is to evaluate whether certain grape varieties originate wines more susceptible to develop volatile phenols. One task of the thesis plan is the chemical characterization of varietal wines regarding the precursor compounds of volatile phenols. For this study, 58 commercial single red wines from 8 different varieties were selected: Aragonez, Carbenet Sauvignon, Castelão, Syrah, Touriga Franca, Touriga Nacional, Trincadeira and Vinhão. The determination of the volatile phenols precursors both on the free form (p-coumaric, ferulic and caffeic acids) and as tartaric esters of hydroxycinnamic acids (caftaric, coutaric and fertaric acids) was performed by liquid phase chromatography (HPLC -DAD, LC-MS). The volatile phenols were also analysed in these wines. The predominant phenolic acid was p-coumaric acid, the highest concentrations being found in Syrah and Touriga Franca and the lowest in Touriga Nacional and Trincadeira. The distribution of tartaric esters of hydroxycinnamic acids was quite homogeneous among the different grape varities. Most of the volatile phenols precursors in Touriga Nacional, Trincadeira and Aragonez are in the combined form thus showing low free/combined ratios.
Wines selected from the Touriga Nacional, Cabernet Sauvignon and Trincadeira varieties were inoculated with one strain of Brettanomyces/Dekkera for assaying the survival capacity in different grape varieties. This strain was previously selected from a group of 18 yeast strains based on the growth and resistance to ethanol at different concentrations (10, 12, 13 and 14% v/v). The results suggest that the grape variety influences the survival behaviour of the yeast. The highest viable counts were found in Trincadeira and the lowest in Touriga Nacional. The precursor compounds and the volatile phenols were monitored in these wines.

Student: Ana Isabel Novo de Figueiredo

Supervisor: Paula Maria Lima Castro

Thesis Tittle: Search of new enzymes for the oxidation of sulfur compounds.

Abstract

Biological oxidation of hydrogen sulfide to sulfate is one of the major reactions of the global sulfur cycle. In the presence of a suitable electron acceptor, bacteria can oxidize hydrogen sulfide, sulfur, sulfite, thiosulfate, and various polythionates under alkaline, neutral, or acidic conditions. The first step of hydrogen sulfide oxidation is its conversion to sulfur or polysulfide in many phototrophic and chemotrophic bacteria by flavocytochrome c, or by sulfide:quinone reductase, which are located in the periplasm and the periplasmic surface of the cytoplasmic membrane, respectively[1,2]. In the last decade our understanding on the acidophilic bacteria has greatly advanced, however knowledge on the physiology and genetics of the heterotrophic sulfur-oxidizing is more scarce, and compared to autotrophic sulfur-oxidizing bacteria these heterotrophs are more versatile[3].

This work aims at the identification of the genes that encode enzyme(s) responsible for H_2S oxidation in the sequenced genome of *Pseudomonas koreensis* A9. This bacterial strain was isolated from an odour biofilter installed in a WWTP, that displayed high sulfide tolerance and growth on with sulfide on concentrations up to 16mM. Activity tests were conducted using crude cell extract as catalyst and measuring the formation of sulfate as the reaction product. Maximum sulfate concentration achieved was 71,6 $mg.L^{-1}$ and a specific activity 2,9 $U.mg^{-1}$ of protein, but when this extract was purified by acetone precipitation and ultrafiltration, results of specific activity reached 67,3 $U.mg^{-1}$ what is very promising compared to the pure sulfide oxidase from *Arthrobacter* sp. (65,1 $U.mg^{-1}$)[4].

Total genomic DNA from strain A9 was sequenced and assembled by a 454-pyrosequencing system Genome Sequencer FLX with GS FLX Titanium. The genome draft was annotated using the Rapid Annotation System Technology (RAST)[5,6]. The sequencing produced 269 031 reads with an average length of 651 bases. DNA sequencing of *P. koreensis A9* and short read *de novo* assembly generated the 6 376 154 bp draft genome with 60,1 % G+C content. The *de novo* read assembly produced 73 contigs with pegs, the longest had 747 025 bases. This genome *encodes 5 738 predicted coding genes distributed by 542 sub-systems from SEED*[6]. Out of 5 738 protein coding genes sequences, 50% were annotated with functional specifications, while the remaining encoded hypothetical proteins. The A9 genome was screened for sulfide oxidation related genes by browsing the RAST subsystems associated

to S metabolism. Functional screening analysis reveals that A9 possesses the genes required for sulfide oxidation to sulfate, with sulfite as the intermediate. The orthologous sequences of these enzymes were found on peg's 3615, peg.2931 and peg. 4936. In addition, rhodanese-like, glutathione:S transferase, thiol peroxidase, among others were identified in the genome which demonstrate the ability and versatility of A9 to sulfide detoxification applications.

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Student: Ana Rita Araújo da Silva Monteiro Monforte

Supervisor: António César da Silva Ferreira; Sara Martins (Unilever R&D) and Rasmus Bro (Copenhagen University)

Thesis Tittle: Chemiomics: A systems chemistry approach to unravel the interface pathways between Oxidation and Maillard mechanism responsible for flavour modulation during wine storage.

Abstract

In foods and in particular in wine and beer, phenylacetaldehyde (PA) is recognized as a key odorant, contributing to “honey” like aroma notes playing a major role on consumers perception of product quality. Strecker degradation (SD) leading to the formation of phenylacetaldehyde (PA) was studied.in wine systems. New insights were gained by using two full factorial designs focusing on the effect of: 1) pH and 2) temperature. In each DoE three factors: glucose, gallic acid and metals at two levels (present or absence) were varied while phenylalanine was kept constant. The obtained results gave a clear indication, with statistical significance, that in wine conditions, the SD occurs in the presence of metals preferentially via the phenolic oxidation independently of the temperature (40°C or 80°C). The reaction of the amino acid with the o-quinone formed by the oxidation of the gallic acid seems to be favoured when compared with the SD promoted by the reaction with α-dicarbonyls formed by MR between glucose and phenylalanine To elucidate the differentially strong impact of glucose on PA formation, a kinetic study has been performed and an important intermediary of the phenolic oxidation reaction: the quinone of gallic acid has been for the first time identified and quantified by LC-ESI-qTOF-HRMS. In the phenolic oxidation model the concentration of o-quinone is the double of the model solution where the two reactions were promoted (phenylalanine + gallic acid + glucose + metals. Results on a real wine system showed that the addition of glucose can have an inhibiting effect on the formation of PA. After 24 hours at 40oC the concentration of PA was higher in the control wine than in the wine with added sugar, 0.74±0.02 and 0.41±0.02 μM, respectively The findings in the present study have given new insights to the understanding of the role of gallic acid, glucose as well as metals in the formation of PA. The DOE results seem to indicate that the presence of metals, is of higher relevance in promoting the Strecker degradation through the gallic acid oxidation pathway at lower pH's and different temperatures. Also, phenolic oxidation alone is the major pathway for phenylacetaldehyde formation, where the role of pH, metals and temperature are the most significant factors. This work has provided further evidence that the presence of glucose in combination with phenylalanine, gallic acid and metals inhibits the PA formation, in both model wine systems and wine itself. By gallic acid quinone quantitation an indication is given that glucose affects directly the concentration of the quinone, which suggest that in white wine glucose has an antioxidant effect by inhibiting o-quinones formation. Overall, the combination of the design of experiments to screen a broad experimental region combined with target experiments showed to be an efficient way to study the impact of reaction parameters on the formation of Strecker aldehydes, and key aroma compounds, in model-wine conditions. An approach that will be further applied in future studies.

Student: Catarina Pereira de Melo Vila Real

Supervisor: Prof. Elisabete Pinto e Prof. Ana Gomes (Co-orientador)

Thesis Tittle: Dietary fibre intake and tailored fermentation for increased availability of cereal fibre-rich food matrices: bridge between Africa and Europe

Abstract

Chronic diseases are the major cause of death and disability worldwide[1]. Whole grains consumption, mainly due to the high content of fibre, are associated with reduced risk of chronic diseases[2–4]. Despite the demonstrated benefits of fibre-rich products, there is fibre inadequacy in Portugal[5]. Furthermore, while data is scarce[6] and no specific recommendations are currently available, it might also be so in African countries. This study proposes a two-fold research Europe-Africa bridge strategy: 1) to study the typical adult diet, focusing on fibre in Kenya, Burkina Faso, Portugal and Finland; food consumption knowledge about these African populations will be increased, and compared with that in the European adult populations; 2) to use major African staple sorghum and millet whole grain[7] and gluten-free[8,9] cereals for the development of innovative fermented products that can contribute to overcome fibre inadequacy in both European and African markets.

Considering the first workfront, two different semi-quantitative food frequency questionnaires (FFQ) were developed to study dietary intake in both African countries. The Kenyan FFQ was validated, using 3 non-consecutive 24-hour recalls as the gold standard, and it was shown to be a valid and reproducible instrument to rank Kenyan urban adults according to their dietary intake. This validated FFQ was used to study the dietary intake of 526 urban Kenyans. These data are under analysis and a paper regarding Kenyan dietary intake is being written. In Burkina Faso, the work is in progress, having all the practical tasks for the validity study been completed, remaining to conclude the analysis of the collected data. Secondary data analysis for Portugal and Finland is also in progress.

In terms of the second research axis of this project, fermentation of whole grain African cereals by different strains is being studied. In a first stage four probiotics strains were used. In this phase cereals were dry- and wet-milled, slurries were prepared in water (1:9) and fermentation was performed at 30 ºC, at 200 rpm, in an orbital incubator. In experiments involving fermentation with *Bifidobacterium* strains, wet milling was placed aside (based on first stage experiments findings) and only dry milling was put in place. In this second phase, fermentation experiments using the indigenous isolates from both Kenya and Burkina Faso were also initiated. Fermentation experiments have followed the same procedure, but in this case fermentation behavior has been studied in the presence and absence of sucrose to test for the possible *in situ* production of exopolysaccharide. The next steps will include the study of the fermentation behavior of co-cultures (the most promising probiotic and indigenous strains) on native cereals and

nutritional impact; the establishment of the final formulation conditions, considering eventual addition of texture and flavour agents and testing of diverse drying technologies for strain survival.

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Student: Débora Andreia Campelo Campos

Supervisor: Professora Maria Manuela Pintado

Thesis Tittle: Development and characterization of functional ingredients through valorization of pineapple by-products: production, bioactivity and potential application

Abstract

The isolation and purification of bioproducts are very important processes in the biotechnology industry, representing 80–90% of total production costs. Furthermore, the development of simple and viable methods for protein purification has been an essential pre-requisite for many advances in biotechnology (Hari Krishna and Karanth 2002).

Bromelain (BR) is a crude, aqueous extract from the stems and immature fruits of pineapples (*Ananas comosus*) and can be found in the tissue of plants of the *Bromeliaceae* family and belongs to cysteine-proteinases family (Rowan, Christopher et al. 1990). Several processes of extraction were developed, but all with some disadvantage (high cost, low purity, high use of chemical products). From an industrial view (low cost of production) the most suitable was the simplest method; a cooled pineapple juice centrifuged, ultrafiltrated and lyophilized, constituting an unusually complex mixture of different type of compounds, interfering with BR bioavailability (Cooreman 1978).

This enzyme offer a wide spectrum of therapeutic efficacies: *in vitro* and *in vivo* antiedemateous, antiinflammatory, antithrombotic and fibrinolytic activities (Maurer 2001), increasing the importance of determining a viable extraction and purification method for this enzyme. A new method of purification and isolation of BR was developed, using a natural food safe polysaccharide.

Carrageenan (Carr) is derived from certain species of red seaweeds (*Rhodophyceae*) and shows a wide range of rheological and functional properties. Previous reports have demonstrated the use of this polysaccharide to isolate and immobilize enzymes (Fabian, Sonenberg et al. 2010).

Using extracts produced from two types of pineapple residues (stems and peels) complex formation of BR-Carr was studied and improved to obtain an isolate of BR by precipitation. Thus, statistical models designs were created. First, was developed a model to exclude non-influent independent variables and narrow ranges, thus in the initial factorial design (Central Composite Design – CCD) were studied four independent variables (protein concentration, polysaccharide concentration, pH and time of contact between protein and polysaccharide) described elsewhere (Phillips and Williams 2009) as influents at protein-polysaccharide complexes formation and three responses evaluated. The independent variables

that presented no effect on the responses were eliminated and ranges were optimized. Box-Behnken Design (BBD) was the followed statistical model design used to optimize the BR, from each extract.

Through the experimental results was possible to concluded that, it was possible to separate and precipitate BR from an aqueous extract maintained its biological activity (yield of enzymatic activity was around 80-90% at precipitate), under a non-soluble complex formation. The statistical models applied determined the optimum of each independent variable of precipitation for each extract.

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Student: Eduardo Manuel Aguiar da Costa

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

Thesis Tittle: *Chitosan in textiles: Towards a greener and functional solution*

Abstract

The textile industry in developed countries is confronting the world's marketing conditions and competitive challenges, which are driving towards the development of advanced, highly functional textiles and textiles with higher added value. The actual usage of polymer and textiles have a vast number of advantages and attractiveness as a material. However, despite these advantages, polymers have limitations, namely the lack of surface properties that are conducent to the development of functionalized textiles. Chitosan being biocompatible, non-immunogenic, non-toxic and easily degradable in the environment makes it one of the most promising compounds for a greener future for textiles. Furthermore, besides its significant biological activity, chitosan shows great affinity towards dyestuffs, opening the window for the incorporation of natural dyes into textiles at affordable prices. One of the possible avenues of application of chitosan in textiles is nanotechnology. Application of chitosan nanoparticles for antimicrobial functionalization of textiles is highly attractive due to the very high surface area to volume ratio and the expected desirable bioactivity even at very low concentrations.

Chitosan high reactivity allows for spontaneous nanoparticle production in the presence of polyanions, such as tripolyphosphate, through a method known as ionic gelation, a method with high reproducibility and facility to be carried out. Furthermore, this method is highly controllable and allows for a certain degree of control of the produced particles size and surface charge. With this in mind we sought to access the impact that the variation of 3 physical characteristics of the process (addition time, reaction time and rotation speed) at two different pH values (4 and 5) had upon the produced particles (through particle size and charge determination) and, if possible, select the best combination of factors. Following this, for the selected condition, the biological potential of the nanoparticles was assessed upon skin related commensal and pathogenic microorganisms in planktonic and sessile settings.

We found that the variation of the nanoparticles physical production parameters had a direct impact upon particle size and charge and that it was possible to define a set of best performing conditions. Nanoparticles produced under this conditions inhibited both the planktonic and sessile growth of various skin related microorganisms with particular relevance to several multidrug resistant, such as MRSA, VRSA and *Acinetobacteri baumannii*. Overall, these results show that it is possible to physically modulate chitosan nanoparticles production through the ionic gelation method and that the produced particles are biologically active upon skin related multidrug resistant microorganism, thusly opening the window for the development of nanoparticle functionalized textile with biomedical potential.

Student: FELIX POMPEYO FERRO MAYHUA

Supervisor: Dra Celia Manaia / Dra. Ivone Vaz-Moreira

Thesis Tittle: Ecology and antimicrobial resistance of *Ralstonia* spp. in the urban water cycle

Abstract

Members of genus *Ralstonia* are ubiquitous in aquatic environments, being the species *R. pickettii* the most frequently reported. Members of this species display resistance phenotypes to different antimicrobials and can be opportunistic pathogens. Previously this project studied isolates of *Ralstonia pickettii* and *R. mannitolilytica* and observed the association of tolerance to antibiotics and metals and the behavior under stress conditions. Aminoglycosides resistance was associated with a highest tolerance to arsenite, being both types of stress capable of enhancing biofilm formation. This effect was observed only in aminoglycoside resistant (AGR) strains but not in the susceptible strains.

In a second phase of this study, 37 *R. pickettii* isolates from hospital effluent (n=6), mineral (n=14), and tap water (n=17), 18 *R. pickettii* isolates artificially evolved in the presence of selective pressures [UV radiation (n=4), and gentamicin (n=14)] were selected to characterize genetic determinants hypothetically related with genetic recombination events leading to antibiotic resistance acquisition in *Ralstonia* spp. The antimicrobial susceptibility phenotypes and genotypes were characterized using the disk diffusion method and the beta-lactamase genes $bla_{\text{OXA-22}}$ and $bla_{\text{OXA-60}}$, genes related with the methylation of 16S rRNA (*rmt*A and *rmt*D) and mutations in the efflux pump gene *cme*A were assessed based on PCR and nucleotide sequencing analyses. The presence of a set of genes characteristic of an integrative conjugative element (ICE) was screened using conventional PCR and the presence, number and size of plasmids was inferred based on Pulsed Field Gel Electrophoresis (PFGE).

For the strains evolved through successive transfers under stress conditions (increasing concentrations of gentamicin), the aminoglycoside-susceptible strain (AGS) showed the potential to acquire resistance to aminoglycosides, while the resistant one kept the original phenotype.

All wild *R. pickettii* strains showed resistance to colistin sulphate, probably due to intrinsic resistance. All the mineral and tap water strains, and some of the wastewater strains presented also resistance phenotype to aminoglycosides, which suggests that this can be an acquired resistance.

With exception of one tap water isolate, all the AGR strains were observed to harbor an ICE, in contrast with the susceptible strains.

Most (n=36) of the wild strains of *R. pickettii* presented at least one plasmid, with sizes ranging 225-300 kbp in mineral water, 180-225 kbp in tap water and 77-340 kbp in wastewater. The evolved strains had

plasmids of 77 and 320, while the respective wild strains presented plasmids of 300-320. These data did not allow the establishment of a relationship between the presence of the plasmid and the tolerance to aminoglycosides.

The data obtained so far suggests that the presence of ICEs may be associated with aminoglycosides resistance however, a more exhaustive analysis of the results and their implications with the antibacterial resistance will still be realized. Other studies exploring the molecular mechanisms of resistance to aminoglycosides and arsenite will be undertaken in the next stages of this project.

Student: Giovânia Carvalho Araújo

Supervisor: Paula Castro

Co-supervisor**:** Nadine Sousa

Thesis Tittle: Symbionts as trees growth promoters under stress conditions

Abstract

The objective of this program is the selection of plant growth promoting rhizobacteria (PGPR) and ectomycorrhizal fungi (ECMF) capable of establishing symbiosis with *Quercus suber* and *Pinus pinea*, to promote better growth and survival in the field after transplantation from greenhouses. For a deeper view of the signaling involved during mycorrhizal establishment, with and without the presence of bacteria, the level of phenolic compounds during mychorrizal establishment was investigated. The selected fungi were *Lactarius delicious*, *Pisolithus tinctorius* and two *Suillus granulatus* isolates. The bacterium chosen was *Bacillus subtilis*, due to its potential PGPR abilities (Pereira & Castro, 2014), and because in initial in vitro assays proved to significantly affect fungal growth (promoting or inhibiting). An in vitro study was carried out to evaluate the mycorrhization process, investigating possible variations in the phenolic compounds caused by the contact of the symbionts with the plant. Using a microcosm setup, germinated plants were placed on sterile substrate where they were inoculated with fungi and/or bacteria, for a total of 7 inoculation treatments plus the non-inoculated control. The systems were maintained in a controlled growth room in a time course experiment in which a destructive sample was performed after 10, 20, 40 and 70 days. Five replicates were analyzed per treatment. Biometric parameters (height, root length, diameter and weight), nutritional status and metabolites in the plant were analyzed by HPLC analysis. The remaining plants were transplanted to two different types of soils from natural areas, one very sandy and the other of the clayey type. After six months, the plants were removed for various analyzes such as biometrics, phenolic compounds and percentage of mycorrhization. Samples were also taken for genetic, enzymatic and edaphic analyzes. The plants grown in the sandy soil presented a reduced growth and low efficient use of nutrients when compared to the ones in clay soil, even though the percentage of mycorrhizas was higher. In general, seedlings inoculated with *S. granulatus* and *Suillus* with *B. subtilis* showed better development than the remaining inoculated plants, especially in sandy soil. In some cases, catechin and protocatecuic acid tend to increase over time, whereas chlorogenic and coumaric acids tend to decrease. Furthermore, the presence of the bacterium with fungus interfered with the level of phenolic compounds in the plant tissue.

Student: Ingrid Collombel

Supervisor: Tim Hogg

Thesis Tittle: Vinification and post vinification micro-ecology of wines; the role of phenolic composition and the effects on quality

Abstract

Wine phenolic compounds can activate or inhibit microbial growth and metabolisms depending on their structure and concentration. Their antimicrobial effect differs according to the species, and the strain among a same species. Most of the actual studies have been directing their work using medium culture and high phenolics´ concentrations. Only few tested the effect of real wine phenolics´ concentrations in wine. The pre-fermentation steps, mostly maceration, regulates the initial phenolics´ concentrations of a wine (skins and stems contact, maceration time, woodships addition, etc.). In this study, groups of phenolics (HBA, flavanols, flavonols, HCA and stilbenes) were added, 2 (*2) or 3 (*3) times their initial concentrations, in a 2016 Douro red wine collected before malolactic fermentation (MLF), inoculated or not (spontaneous) with *Oenococcus oeni* Oenos. In both MLF (spontaneous and initiated), 2 controls (pasteurized or not) without addition of phenolics were used for comparisons. Chemical and microbial characteristics of the wines were followed along MLF (start 0d, mid-term 14d, end 28d), as well as 3 and 5 ½ months after inoculation. HPLC and GC methods were used for the analysis of the metabolites composition of the samples (sugars, organic acids, phenolics, amino acids and volatiles). Microbial growth was measured using the drop-count technique. All the wine samples were replicated. Extra samples were frozen for later possible metabarcoding (16S and ITS for microbial identification) and metagenomics (shotgun for microbial functional aspects) analysis. The initial phenolics raises, as predicted, have different impacts on the microbial and metabolites compositions of the samples, according to the type and stage of MLF. Among several interesting results can be listed, the bacterial growth inhibition by flavonols*3, and HCA at the mid-MLF. After 28 days of MLF, resveratrol*3 (stilbene), activates the bacterial growth, when flavanols, in the contrary, inhibit it. Three months after the initiation of MLF, a new *Lactobacillus* appears, inhibited by HCA*3. In the non-inoculated wine, the organic acids metabolisms are affected by resveratrol*3 and HCA*3. Resveratrol*3 activates the acetic acid production, toward the reduction of the lactic acid production. HCA*3 inhibit the lactic acid production too, although, probably linked to its antibacterial function. The addition of coumaric and ferulic (HCA) induces the release of caffeic, not necessary from its esterified form (caftaric acid). The chemical anthocyanins´ reduction is emphasized by resveratrol*3 and HCA*3, after 3 months of MLF. Flavanols*3, on the other hand, repress surprisingly the chemical decrease of cyanidin-3-0-acetylglucoside along MLF. Concerning the volatiles, decanoic and dodecanoic acids evolution is intriguing. HCA*2 highlight the reduction of these 2 acids at mid-MLF for non-inoculated wines and after 28 days for inoculated ones.
Manuscripts for publication in preparation.

Student: Isabella Ribeiro de Figueiredo Vieira

Supervisors: Margarida Silva, Elisabete Pinto, Teresa Brandão

Thesis Title: Biotechnology for supper: perceptions, concerns and attitudes of health professionals towards genetically modified food

Abstract

The project *Biotechnology for supper: perceptions, concerns and attitudes of health professionals towards genetically modified food* (GMF) aims to characterize the positioning of medical doctors and nutritionists regarding GMF, in order to identify significant gaps in knowledge and solutions to improve future training. For the study social science methodologies are used: surveys, focus groups and interviews. The main data collection instrument was created, tested and applied. Thus, the efforts to date consisted in elaborating a questionnaire and structuring a group focus and interview scripts. The semi-structured questionnaire was developed, disseminated by multiple channels among physicians and nutritionists, and more than 500 responses were obtained. There are several genetically modified foods currently on the Portuguese market, such as soybean oil and corn flour. Officially they are all safe, but contradictory information has been published in scientific journals. It is likely that doctors and nutritionists have already reflected on the subject but their positioning has not been studied. The GMF survey focused on personal opinions, individual consumption, formal education on the topic, importance in professional practice, professional decisions and basic knowledge. Results indicate that women hold a less favorable opinion regarding GMF than men. Age does not appear to be a factor. Results also show that physicians, in general, are not sure about the impacts of transgenic foods on health. On the nutritionists' side, many neither agree nor disagree with with GMF, although they consider as necessary both a debate and formal study on GMF's health implications. It is to be expected that health professionals be faced with questions from concerned patients. A lack of proper training, however, can prevent them from fully and confidently addressing those concerns. These results have been presented at scientific meetings. At the moment, statistical analyses (Multiple Correspondence Analysis) are being carried out in order to identify typical profiles and better characterize these professionals. Future activities include the development of a training proposal to be inserted in university curricula. This will be done in cooperation with the relevant institutions, whose course coordinators are to be interviewed.

Student: Manuel João Rebelo Araújo Oliveira

Supervisor: Susana Carvalho and Marta Vasconcelos

Thesis Tittle: New challenges on the control of Flavescence dorée in grapevine: exploiting genetic resources and the use of elicitors

Abstract

Flavescence Doreé (FD) is a quarantine disease caused by a phytoplasma and transmitted to healthy plants by an insect vector, *Scaphoideus titanus*. This disease affects grapevines and is a serious problem to the stability and sustainability of the Portuguese wine industry, since it can lead to drastic harvest losses and to the death of the infected plants. To date there is no effective treatment against FD. This study, conducted at Quinta do Corvo (Fafe), aimed at exploring the morphological, physiological and genetic differences between heathy grapevines cv. Loureiro (with 20 years) and those ones infected with FD. Moreover, it was also tested the effect of the plant defence elicitor Methyl Jasmonate (MeJA 12.5 mM and 25 mM) on these parameters. Sprouting percentage, fertility rate, SPAD, leaf area, quality parameters (total acidity, brix, probable alcohol content), productivity, gene expression were evaluated and transmission electron microscopy (TEM) was carried out. This study concludes that the disease delayed plant development when compared to healthy plants (on average 15 days), and led to production losses (66%). Still, it was possible to visualize with TEM the changes and disorganization of phloem cells, showing an accumulation of callose in the sieve elements and necrotic cells. Finally, it was evidenced that MeJA played an important role in activating signalling pathways, because an increase was registered after the application with the elicitors in some genes.

Keywords: Flavescence Doreé, phytoplasma, *Scaphoideus titanus*, methyl jasmonate, gene expression.

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Thesis Tittle: Utilization of plant-growth promoting bacteria (PGPB) to ameliorate iron nutrition in the legumes

Abstract

Legumes are important crops for human food and animal feed, having a positive impact on environmental health. Iron (Fe) deficiency chlorosis (IDC) is a serious nutritional problem affecting legumes, particularly when grown in calcareous soils. Recent evidence suggests that the utilization of biofertilizers enriched with plant-growth promoting bacteria (PGPB) may be an efficient strategy for enhancing iron nutrition in these crops. In this project we aim at understanding the impact of PGPB as IDC control agents through morphological, physiological and molecular analysis in soybean and common bean.

Twenty four PGPB isolates from a CBQF collection were first selected based on ammonia and siderophore production, and phosphate solubilization, and tested for *in vitro* traits related to Fe nutrition (e.g., organic acid production and Fe reduction). Three isolates were further selected based on the best capacity to reduce Fe(II). In parallel, 76 bacteria were isolated from shoots (18%) and roots (53%) of field grown soybean plants, and from rhizospheric soil (29%), 45 of which were identified based on the 16S rRNA gene sequence analysis. Results show that *Bacillus*, *Kocuria*, *Methylobacterium*, *Pseudoclavibacter* and *Staphylococcus* genus were isolated from shoots; *Agrococcus*, *Aliihoeflea*, *Alishewanella*, *Bacillus*, *Bosea*, *Brachybacterium*, *Chryseobacterium*, *Dyadobacter*, *Flavobacterium*, *Microbacterium*, *Paenibacillus*, *Pseudomonas*, *Sphingobacterium* and *Staphylococcus* genus were found in roots; and *Bacillus, Chlamydia*, *Kocuria*, *Microbacterium*, *Ochrobactrum*, *Paenibacillus* and *Sporosarcina* were isolated from soil. The *Bacillus* genus was present in the three isolation sites and a greater bacterial diversity was found in the soybean roots. *Methylobacterium*, *Pseudoclavibacter* and *Staphylococcus* genus were only present in shoots; and *Chlamydia*, *Ochrobactrum* and *Sporosarcina* genus were only isolated from the rhizospheric soil.

The bacterial PGP abilities of these isolates will also be tested applying techniques currently used in CBQF (amount of IAA, ACC-deaminase activity, phosphate solubilization, and ammonia and siderophore production) and those with the best PGP abilities will be tested for the same analysis related to Fe nutrition already performed to the CBQF isolates.

The best candidates selected within the CBQF collection and soybean isolates are being used in a soil experiment, in order to assess the effect of bacteria inoculation on IDC symptoms alleviation. Three inoculation treatments were tested: soil inoculation, seed inoculation and a combination of both. Inoculations were performed in a calcareous soil (pH 7.4) from the North of Portugal. Plants will be evaluated for several IDC and plant growth parameters, such as: visual IDC score, plant height, root length, SPAD, leaf area, chlorophyll content, lipid peroxidation, mineral content and expression of genes related to iron nutrition and PGPB colonization.

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Thesis Tittle: Development and characterization of functional ingredients from olive pomace: bioactivity and potential application as bioactive edible films

Abstract

Olive production is a major agricultural sector in the Mediterranean area, including Portugal. The production of olive oil generates 15-35% of semi-solid waste, known as olive pomace (OP). The current options for its treatment (energy pathways, landfill or fertilizer) reveal operational and environmental weaknesses. These options also represent a wastage of OP bioactive compounds. The recovery of OP bioactive compounds has been exploited mainly regarding polyphenols, despite the potential of other nutrients like polysaccharides and proteins. As well, proteins and polysaccharides are generally studied separately from polyphenols, but there is scientific evidence of a strong association of these compounds and phenolics. Since only 2% of polyphenolics of olive are extracted from olive oil, the OP can be a great source of polysaccharides and proteins combined with powerful antioxidants.

The main goal of this work is to develop “zero” waste valorisation strategy for OP, applying more sustainable and economically viable processes to recovery high added value bioactive and functional compounds with the final goal of achieve new food grade ingredients and edible films/coatings.

The OP has been collected in two olive mills of Inner Centre of Portugal and stored on -80°C. The studies performed during the last year were focused in the general composition (moisture, ash, protein, crude fibre) of the collected OP and its factions – Aqueous Phase (AP-OP) and Pulp (P-OP) - using standard AOAC methods. Centrifugation, drying, milling and sieving (<1mm) were applied to obtain the different fractions. The quantification of total free (TFPC) and bound polyphenols (TBPC) by the Folin-Ciocalteu method and determination of antioxidant activity by ABTS and DPPH were also performed. The whole OP samples only showed significant differences in terms of moisture (68-78%, in wet weight) and fat (16-20%, in dry weight), which can be explained by possible differences in olive oil extraction process. Crude fibre (36-38% dw) is the major component and protein represents only approximately 9% (dw). The results of TFPC, TPBC, ABTS and DPPH achieved were in agreement with the literature, confirming the high bioactivity of OP. The identification of phenolic compounds by HPLC-DAD in the fractions and in the whole OP was also performed. The results of general composition analysis and evaluation of TFPC, TPBC and antioxidant activity of AP-OP and P-OP have allowed realize the possibility of exploration of these fractions. The influence of drying temperature on pulp fraction was also investigated. The AP-OP was dried at different temperatures (50, 70 e 90 ºC) and sieved (>400 µm to <150 µm) to obtain bioactive

powders (non-extractive approach). At the moment, the general composition and bioactivity of different sub-fractions of pulp are under study. The extraction of compounds of interest using different eco-friendlier technologies in the fractions obtained will be the focus of the future work.