

Project Title: The role of suberin degradation in signaling morphogenesis/development during fungal saprophytic and pathogenic life-cycles

Project area: Fungal biology

Supervisor: Doctor Cristina Silva Pereira and Dr. Isabel Martins

(<http://aem.itqb.unl.pt/>)

Duration: 9 to 12 months

Host institution: ITQB

Number of students: 1

Summary

Land plants have evolved through >400 million years, developing functional polyester-based defensive structures, such as suberin and cutin, that limit pathogen adhesion and invasion and play roles in mediating fungi/plant interactions. Recently, we demonstrated that suberin elicits both sexual development and stress responses in *Aspergillus nidulans*. Its degradation/utilisation involves unknown regulatory elements besides the known elements of fatty acid utilisation. Preliminary observations showed also that the lipid-monomers released strongly inhibited conidia germination, contrary to those derived from plant emulsified fatty acids.

Understanding the genetics of the interactions between plants and both, phytopathogenic and saprophytic fungi, particularly with polyester-based defensive structures, is vital for developing effective management strategies for limiting/controlling fungal invasion. The research plan will essentially focus on *Aspergillus nidulans* (saprophytic) and *Fusarium oxysporum* (phytopathogenic).

The project plan will focus on

i) Elucidate the transcriptional regulation of suberin degradation. Gene expression assays of targeted genes during degradation of suberin (or other natural or synthetic polyesters) in both wild type and deletion mutant strains will be performed. Gene shift assays will be used to identify putative effectors of cutinase/lipase genes.

ii) Identify suberin monomers signalling in fungal morphogenesis/development. Suberin monomers (viz. released during fungal growth or chemically/enzymatically hydrolysed) able to trigger morphological/developmental changes (and the expression of cutinase/lipase genes) will be identified combining standard cultivation assays, LIVE/DEAD methods and chemical assays.

SHORT CV

PERSONAL DATA

Name: **Cristina** Maria da Costa **Silva Pereira**

Date of birth: 16th May 1973; Married, one son; Portuguese

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Synopsis: I am since 2008, four years after completion of my doctoral studies, the head of the Applied and Environmental Mycology group at ITQB (<http://aem.itqb.unl.pt/>). My team currently encompasses six PhD students, two postdoctoral scientists, one technician and two research fellows, all of whom are under my sole or principal supervision. I have supervised completion of two ITQB doctorates (2011 and 2013) and co-supervised one UWS (UK) doctorate (2012). I lead two international consortia (EEA and NATO-sfp grants) and national I&D projects.

I have visiting academic post at the Queen's University Ionic Liquid Laboratories and at the University of the West of Scotland (UK) and a number of long standing national and international collaborations. In 2007, I was awarded the prestigious national *Professor António Xavier* memorial award for young scientists, and in 2011 I was selected for a second round of review (interview) by the ERC Starting Grant panel.

I have co-authored 25 peer-reviewed journals, of which I am the corresponding author on 13, plus two peer-reviewed contributions to a Wiley book on Ionic Liquids and to a Springer Encyclopaedia, plus five presentations as a key speaker at international conferences. I am in the executive committee of the Congress on Ionic Liquids 2015.

ACADEMIC QUALIFICATIONS

2004: PhD in Biochemistry, ITQB-UNL, Portugal (July 2004)

1996: Applied Chemistry, Major Biotechnology, FCT-UNL

PROFESSIONAL EXPERIENCE

2008- Assistant researcher at ITQB, Head of the Applied and Environmental Mycology Group

2012- Invited Professor at UWS, UK

2010- Invited Research at QUILL, UK

2004- Principal investigator in research funded consortia, with a cumulative income of near € 2 M.

2007-2008 Postdoctoral Fellowship, FCT, Mix program Portugal and UK (ITQB and QUILL)

2004-2007 Postdoctoral Fellowship, FCT, Mix program Portugal and UK (ITQB/IBET and UWS)

1999-2004 PhD Fellowship, FCT, Mix program Portugal and UK (ITQB, John Innes and IFR)

1997-1999 Research fellowship (IBET, Portugal)

TRAINING EXPERIENCE (ACTUAL TEAM): I am currently supervising 2 postdoctoral fellows; 1 technician; 6 PhD students and 2 research fellows. I have supervised completion of 2 ITQB doctorates (2011 and 2013) and co-supervised one UWS doctorate/2012 (UK). Three temporary research fellows since 2008.

SELECTED ACTIVITIES: I **lecture** "Biocatalysis" in the ITQB PhD course (2008-2013), and co-coordinate the Biotechnology module (2011-2013). I have attended EMBO (2009) and ProSciencia (2008) **management courses**. I was in the **executive committee** of the Congress on Ionic Liquids 2015 and in the **organising committee** of Workshop Ciência 2007 (Rectory of the UNL, 2008). I have been **awarded** with: PhD Fellowship, FCT, 1999; Mix Program Portugal and UK (ITQB, John Innes and IFR);

two Postdoctoral Fellowship, FCT (2004 and 2007) and with the Young scientist *Professor António Xavier* award (2007)

FUNDED PROJECTS (personal cumulative income in externally financed projects ~ € 2 M)

1. *Soil function profiling during fungal bioremediation: integrated bio-geochemical and meta-proteomics assessment*, Portuguese Science Foundation PTDC/AAC-CLI/119100/2010 (2012-2014). **COORDINATOR**. Team budget € 150k (€ 185k).
2. *Creating value from bio-wastes: suberin extraction and biotransformation in biocompatible ionic liquids aiming on novel biomaterials and compounds*, Portuguese Science Foundation PTDC/QUI-QUI/120982/2010 (2012-2014). **COORDINATOR**. Team budget € 50k (€ 85k).
3. *Bio-cement: Suberin in structural composites, QREN, I&DT project* (2013-2014). Coordinated by ESLAM. Team budget € 300k.
4. Bilateral cooperation: DAAD N° A-19/11 (ITQB and Regensburg University) (mission costs).
5. *Waste bio-elimination, using genetically manipulated fungal species in an ionic liquid environment*, EEA grant mechanism¹, PT015 (ended April 2011). **COORDINATOR**. Team budget € 600k (€ 1.2M over 4 years).
6. *Integrated Isolation, Bio- and Organic-Synthetic Transformations of Portuguese Natural Resources*, Portuguese Science Foundation PTDC/QUI/73061/2006/FCT (ended December 2011). Collaborative project. Team budget € 30k.
7. *Preventive and remediation strategies for continuous elimination of PCPs from forest soil*, Sfp-NATO (ended June, 2011). **COORDINATOR**. Team allocated budget € 34.5k (€311k).
8. *Bioremediation of PCPs by the co-metabolism of cork indigenous fungi*, Portuguese Science Foundation. POCTI/AMB/57374/2004/ FCT (ended December 2009). **COORDINATOR**. Team allocated budget € 54k.
9. Bilateral cooperation: CRUP N. B-34/07 (ITQB and QUILL) and N. B-20/06 (ITQB and UWS) British cooperation grants.

Pending applications: *Development of novel antimicrobial biomaterials for chronic wound healing*. ERC-2013-SyG (Corresponding PI)

PEER REVIEWD PUBLICATIONS

Referred articles in journals (*, corresponding author)

27- I Martins, H Garcia; A varela, O Nunez, S Planchon, MT Galcerans, J Renaut, LPN Rebelo and **C Silva Pereira***, Enlightening the mechanisms behind *Aspergillus nidulans* capacity to degrade cork cell walls using a secretome analysis. Submitted

26- I Martins, DO Hartmann, PC Alves, S Planchon, J Renaut, MC Leitão, LPN Rebelo and **C Silva Pereira***, Proteomic alterations induced by ionic liquids in *Aspergillus nidulans* and *Neurospora crass*. Submitted

25- CM Visagie, J Houbraken, C. Rodrigues, **C Silva Pereira**, J. Dijksterhuis, KA Seifert, K Jacobs, RA Samson. Five new *Penicillium* species in section *Sclerotiora*: a tribute to the Royal Dutch family. *Persoonia*. *accepted*

24- DO Hartmann and **C Silva Pereira***. A molecular analysis of the toxicity of alkyltributylphosphonium chlorides in *Aspergillus nidulans*. *New J. Chem.* 2013. NJ-ART-02-2013-000167, DOI: 10.1039/C3NJ00167A

¹ <http://www.eeagrants.org.pt/eeagrants/index.aspx>

- 23- R Ferreira, H Garcia, AF Sousa, CSR Freire, AJD Silvestre, LPN Rebelo, **C. Silva Pereira***. Isolation of suberin from birch outer bark and cork using ionic liquids: a new source of macromonomers. *Industrial Crop and Products*. 2013, 44:520-527.
- 22- MB Carvalho, J Medeiros, I Martins, S Planchon, J Renaut, O Núñez, H Gallart-Ayalla, MT Galceran, A. Hursthouse, **C. Silva Pereira***. The response of *Mucor plumbeus* to pentachlorophenol: a toxicoproteomics study. *J Proteomics*. 2012, 78:159-171.
- 21- LMT Frija, H Garcia, C Rodrigues, I Martins, NR Candeias, V André, MT Duarte, **C Silva Pereira*** and CAM Afonso*. Short Synthesis of the Natural Product 3 β -Hydroxy-labd-8(17)-en-15-oic Acid via Microbial Transformation of Labdanolic Acid. *Phyto Chemical Letters*. 2013, 6:165-169.
- 20- R Ferreira, H Garcia, AF Sousa, M Petkovic, P. Lamosa, CSR Freire, AJD Silvestre, LPN Rebelo, **C. Silva Pereira***. Suberin isolation process from cork using ionic liquids: characterisation of ensuing products. *New J. Chem*. 2012, 36 (10), 2014 - 2024
- 19- M. Petkovic, D. Hartmann, G. Adamova, K. Seddon, LPN Rebelo and **C. Silva Pereira***. Unravelling the mechanism of toxicity of alkyltributylphosphonium chlorides in *Aspergillus nidulans* conidia”, *New Journal of Chemistry*. 2012, 36, 56-63
- 18- MB Carvalho, S Tavares, J Medeiros, O Núñez, H Gallart-Ayalla, MC Leitão MT Galceran, A Hursthouse and **C. Silva Pereira***. Degradation pathway of pentachlorophenol by *Mucor plumbeus* involves phase II conjugation and oxidation-reduction reactions. *J. Hazard. Mat*. 2011, 198, 133-142.
- 17- **C Silva Pereira**, JML Ribeiro, AD Vatulescu, K Findlay, AJ MacDougall, PAP Jackson*. Extensin network formation in *Vitis vinifera callus* is an essential and causal event in rapid and H₂O₂-induced reduction in primary cell wall hydration. *BMC Plant Biology*. 2011, 11(106), DOI: 10.1186/1471-2229-11-106.
- 16- M Petkovic, KR Seddon, LPN Rebelo and **C Silva Pereira***. Ionic Liquids: A Pathway to Environmental Acceptability. *Chem Soc. Rev*. 2011, 40 (3): 1383-1403.
- 15- F Deive, A Rodríguez, MC Leitão, A Varela, C Rodrigues, J Houpraken, AB Pereiro, MA Longo, MA Sanromán, R. Samson, LPN Rebelo and **C Silva Pereira***. Impact of ionic liquids on extreme microbial biotypes from soil. *Green Chem*. 2011, 13 (3): 687-696.
- 14- H Garcia, R Ferreira, M Petkovic, J Ferguson, HQN Gunaratne, KR Seddon, LPN Rebelo and **C Silva Pereira***. Dissolution of cork biopolymers by biocompatible ionic liquids. *Green Chem*. 2010, 12: 367-369.
- 13- M Petkovic, J Ferguson, HQN. Gunaratne, R Ferreira, MC Leitão, KR Seddon, LPN Rebelo and **C Silva Pereira***. Novel biocompatible cholinium-based ionic liquids - toxicity and biodegradability. *Green Chem*. 2010, 12: 643-649.
- 12- MB Carvalho; I Martins; MC Leitão; H Garcia; C Rodrigues; MV San Romão, I McLellan, A Hursthouse and **C Silva Pereira***. Screening pentachlorophenol degradation ability by environmental fungi strains belonging to the phylum *Ascomycota* and *Zygomycota*. *J. Ind. Microbiol*. 2009, 36: 1249-1256.
- 11- M Petkovic, J Ferguson, A Bohn, J Trindade, I Martins, MB Carvalho, MC Leitão, C Rodrigues, H Garcia, R Ferreira, KR Seddon, LPN Rebelo and **C Silva Pereira***. Exploring fungal activity in the presence of ionic liquids. *Green Chem*. 2009, 11. 889–894.
- 10- I.S. McLellan, M. Carvalho, I. Martins, **C. Silva Pereira**, A.S. Hursthouse*, C. Morrison, P. Tatner, M.C. Leitão and M.V. San Romão. Polychlorinated phenols and cork forest ecosystems: A review. *J. Environ. Monit*. 2007, 9(10): 1055-1063

- 9- M.C. Basílio, R. Gaspar, **C. Silva Pereira** and M.V. San Romão*. *Penicillium glabrum* cork colonising isolates - preliminary analysis of their genomic similarity. Rev Iberoam Micol. 2006, 23 (3):151-154
- 8- JM Ribeiro, **C Silva Pereira**, NC Soares, AM Vieira, JA Feijó and PAP Jackson*. The contribution of extensin network formation to rapid, H₂O₂-mediated increases in grapevine *callus* wall resistance to fungal lytic enzymes. J. Exp. Bot. 2006, 57(9): 2025-2035.
- 7- **C Silva Pereira**, GAM Soares, AC Oliveira, ME Rosa, H Pereira, N Moreno and MVS Romão*. Effect of fungal colonization on mechanical performance of cork. Int. Biodeterior. Biodegrad. 2006, 57(4): 244-250.
- 6- A.C. Oliveira, C.M. Peres, J.M. Correia Pires, **C. Silva Pereira**, S. Vitorino, J.J. Figueiredo Marques, M. T. Barreto Crespo and M.V. San Romão*. Cork stoppers industry: defining appropriate mould colonisation. Microbiol Res. 2003, 158:1-8.
- 5- P Jackson*, C Galinha, **C Silva Pereira**, A Fortunato, NC Soares, S Amâncio and CP Ricardo. Rapid Deposition of Extensin during the elicitation of Grapevine *callus* cultures is specifically catalyzed by a 40 KDa Peroxidase. Plant Physiology. 2001, 27: 1065-1076.
- 4- **C Silva Pereira**, JJ Figueiredo Marques and MV San Romão*. Cork taint in wine: scientific knowledge and public perception: a critical review. C. Rev. Microbiol. 2000, 26 (3):147-162.
- 3- C. Kopke, A. Cristovão, A.M. Prata, **C. Silva Pereira**, J.J. Figueiredo Marques and M.V. San Romão*. Microbiological control of wine: the application of epifluorescence microscopy method as a rapid technique. Food Microbiology, 2000, 17, 3:241-250.
- 2- **C Silva Pereira**, A Pires, ML Valle, L Vilas-Boas, JJ Figueiredo Marques and MV San Romão*. Role of *Chrysonilia sitophila* on the quality for cork stoppers for sealing wine bottle. J. Ind. Microb. Biotech. 2000, 24:256-261.
- 1- **C. Silva Pereira**, P. Danesh, J. J. Figueiredo Marques and M. V. San Romão*. O gosto a rolha em vinhos- estado actual dos conhecimentos. Ciência e Técnica Vitivinícola, 1999, 14 (2):47-67.

Selected referred book chapters

- M Petkovic and **C Silva Pereira***. Pioneering biological processes in the presence of ionic liquids: the potential of filamentous fungi, in “Ionic Liquids UnCOILed”, ed. K. R. Seddon, R. D. Rogers, N. V. Plechkova, Wiley, 2013.
- M. Petkovic, JL Ferguson, A Bohn, J Trindade, I Martins, MB Carvalho, MC Leitão, Rodrigues, H Garcia, R Ferreira, KR Seddon, LPN Rebelo and **C Silva Pereira***. On the Merge of Fungal Activity with Ionic Liquids towards the Development of New Biotechnological Processes, in Ionic Liquid Applications: Pharmaceuticals, Therapeutics, and Biotechnology, ed. S. V. Malhotra, American Chemical Society, 2010, 115-134