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HOTEL PORTO PALÁCIO

Symposium H: Biotechnology

Poster Communications

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A. Conde, A. Regalado, H. Gerós and M. Chaves
- P H2** Isolation and phylogenetic analysis of two actin genes from *Phytophthora cinnamomi*
Lurdes Jorge, T. Dias, M. Andrade, M. Vaz, A. Dominguez, Altino Choupina
- P H3** Isolation and sequence analysis of alpha-tubulin gene from *Phytophthora cinnamomi*
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- P H4** Characterization of transglutaminase elicitor precursor from plants pathogen *Phytophthora cinnamomi*
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- P H6** Phenotypic profile of *Candida albicans* strains with an artificially altered genetic code
Ana Rita Bezerra, João Simões, Tobias Weil and Manuel Santos
- P H7** Biodegradation of textile azo dyes by a facultative *Staphylococcus arlettae* strain VN-11 using a sequential microaerophilic/aerobic process
Andrea Zille, E Franciscon, F Dias Guimaro, C Ragagnin de Menezes, L R Durrant, A Cavaco-Paulo
- P H8** Isolation and characterization of microorganisms able to degrade the herbicide Propanil and its recalcitrant metabolite 3,4-dichloroaniline
A. L. Gonçalves, C. I. Santos, C. Faria, A. R. Lopes, O. C. Nunes
- P H9** Serum indices: What is its utility?
Isabel Cachapuz, P. Pinto, C. Teixeira, T. Silva, V. Alves
- P H10** *In vitro* oxidative stress markers define the *ex vitro* acclimatization of plantain plantlets previously micropropagated by temporary immersion bioreactor
C. Aragón, L. Carvalho, J. González, M. Escalona, S. Amâncio
- P H11** Connecting photorespiration and *AOX1a* expression in Arabidopsis plants under water deficit
Cristina Cruz, Dave Prinxeton, Anabela Bernardes da Silva, Ana Rita Matos, Jorge Marques da Silva, Maria Celeste Arrabaça and João Daniel Arrabaça
- P H12** Analytical approach for the determination of antiepileptic drugs in human plasma
Geraldes V., Barroso M., Queiroz J.A., Gallardo E
- P H13** Biochemical valorisation of olive oil by-products
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- P H14** *Saccharomyces cerevisiae*, a potential tool to identify molecular biomarkers relevant for the assessment of pesticide toxicity
Fátima N. Gil, Alina Gonçalves, Jörg D. Becker, Cristina A. Viegas
- P H15** Effect of high-temperature on sugar transport in grape cells
Henrique Noronha, A. Conde, N. Fontes, S. Paivae H. Gerós

Characterization of transglutaminase elicitor precursor from plants pathogen *Phytophthora cinnamomi*

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The oomycetes form a phylogenetically distinct group of eukaryotic microorganisms that includes some of the most notorious pathogens of plants. Among these, members of the genus *Phytophthora* cause enormous economic losses on crop species as well as environmental damage in natural ecosystems. *Phytophthora cinnamomi* is the most widely distributed *Phytophthora* species, with nearly 1000 host species.

Transglutaminases are a family of enzymes (EC 2.3.2.13) that catalyze the formation of a covalent bond between a free amine group and the gamma-carboxamid group of protein-or peptide-bound glutamine. Bonds formed by transglutaminase exhibit high resistance to proteolytic degradation. Transglutaminases form extensively cross-linked, generally insoluble protein polymers. These biological polymers are indispensable for the organism to create barriers and stable structures.

Transglutaminases are widely distributed in various organs, tissues and body fluids. The best known transglutaminase is blood coagulation factor XIII, a plasma tetrameric protein composed of two catalytic A subunits and two non-catalytic B subunits. Factor XIII is responsible for cross-linking fibrin chains, thus stabilizing the fibrin clot.

The extremely high cost of transglutaminase of animal origin has hampered its wider application and has initiated efforts to find an enzyme of microbial origin. Since the early 1990s, many microbial transglutaminase-producing strains have been found, and production processes have been optimized. This

has resulted in a rapidly increasing number of applications of transglutaminase in the food sector. However, applications of microbial transglutaminase in other sectors have been explored to a much lesser extent.

Transglutaminases structural sequences with elicitors activity, associated to plant defense mechanisms, were isolated and characterized in *Phytophthora sojae*, *Phytophthora megasperma* and *Phytophthora infestans*.

In this work we describe a method for thermal asymmetric interlaced-PCR, a hemispecific PCR amplification protocol that combines nested, insertion-specific primers (designed in highly conserved region of *Phytophthora* transglutaminases), with degenerate primers, to amplify DNA flanking a known sequence using genomic *Phytophthora cinnamomi* DNA as template.

In this process we sequenced a 2218 bp DNA fragment, that encodes a 533 aa protein which includes an ORF with high homology with *Phytophthora sojae* (70%), *Phytophthora megasperma* (70%) and *Phytophthora infestans* (61%) transglutaminases besides a deduced similar structure.

The homology of our protein (>60%) with transglutaminases of other organisms of the genus *Phytophthora*, allowed us to conclude that our protein has transglutaminase properties.

The analysis of this gene expression by Real Time PCR, in order to amplify and simultaneously quantify the DNA molecule, and Northern Blot Hybridization, using *P.cinnamomi* RNA, obtained in different growth media and infections of *Castanea sativa*, is underway in our laboratory.

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