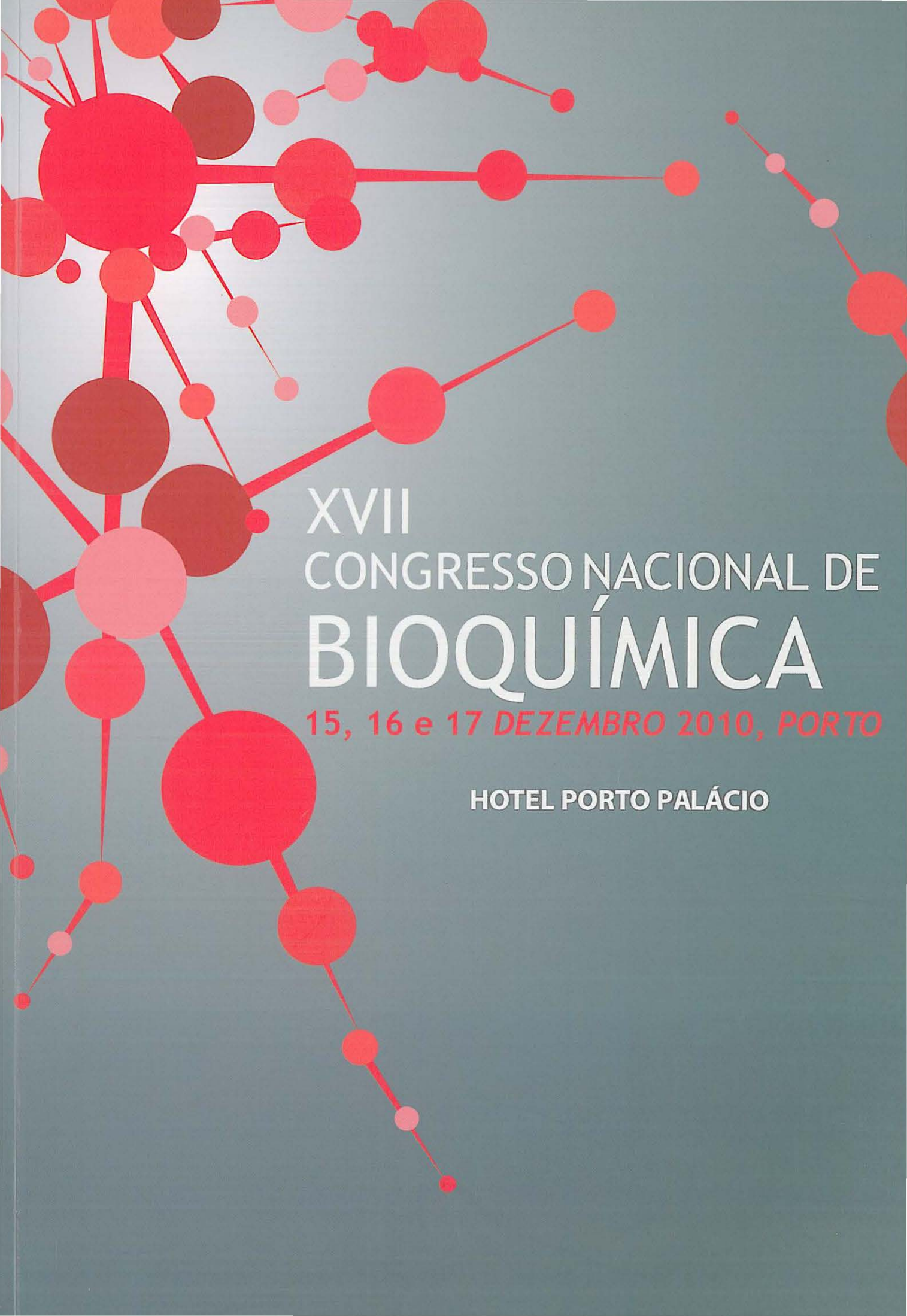

XVII CONGRESSO NACIONAL DE BIOQUÍMICA

15, 16 e 17 *DEZEMBRO* 2010, *PORTO*

HOTEL PORTO PALÁCIO

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Isolation and phylogenetic analysis of two actin genes from *Phytophthora cinnamomi*

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Actins, as the essential component of cellular microfilament, are ubiquitous and highly conserved proteins that play key roles in several basic functions of organism such as cytoskeleton morphology, cell division, cell motility, cellular signal transduction, cellular interaction and organelle movements, as well as locomotion, phagocytosis, endocytosis and exocytosis. Actins are highly conserved structural proteins, found in all eukaryotes. So, actin gene sequences are used as tools in scientific research, for example, for phylogenetic analysis.

Actin in Phytophthora infestans is encoded by at least two genes, in contrast to unicellular and filamentous fungi (Candida albicans, Saccharomyces cerevisiae, Schizosaccharomyces pombe, Kluyveromyces lactis and Filobasidiella neoformans) where there is a single gene. These genes (designated actA and actB) have been isolated from a genomic library of P. infestans. Phytophthora cinnamomi is a host-nonspecific, soilborne, pathogen of many plant species. In Portugal it is most important as a pathogen of chestnut trees.

The purpose of this study was to clone and determine the phylogenetic retionships evidencided by *Phytophthora cinnamomi* actins.

In order to isolate the actin genes, *P. cinnamomi* was grown in cellophane-PDA medium and genomic DNA was used as a template in PCR amplification reactions combining degenerate

primers Act1, Act2, Act3 and Act4. PCR fragments were purified, cloned into pGEM-T vector and transformants were selected. Complete open reading frames (ORFs) of *act1* and *act2* genes were achieved by HE-TAIL PCR, and submitted to EMBL databases (Accession numbers AM412175.1 and AM412176.1).

Act1 has an 1128bp ORF, encoding a deduced protein of 375aa and 41,972kDa. *Act2* ORF has 1083bp and encodes a deduced protein of 360aa and 40,237kDa. Deduced amino acid sequences were analyzed using FASTA programs from EMBL databases. *Act1* showed a 98.9% identity with *P. melonis* actB, 94.4% with *P. megasperma* actin and 96.0% with *P. infestans* actin2. *Act2* showed a 98.9% identity with *Pythium splendens* actin and 98.6% with *P. brassicae* actinA.

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