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Two-component signal transduction in *Agaricus bisporus*: A comparative genomic analysis with other basidiomycetes through the web-based tool BASID2CS

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ABSTRACT

Two-component systems (TCSs) are signal transduction mechanisms present in many eukaryotes, including fungi that play essential roles in the regulation of several cellular functions and responses. In this study, we carry out a genomic analysis of the TCS proteins in two varieties of the white button mushroom *Agaricus bisporus*. The genomes of both *A. bisporus* varieties contain eight genes coding for TCS proteins, which include four hybrid Histidine Kinases (HKs), a single histidine-containing phosphotransfer (HPt) protein and three Response Regulators (RRs). Comparison of the TCS proteins among *A. bisporus* and the sequenced basidiomycetes showed a conserved core complement of five TCS proteins including the Tco1/Nik1 hybrid HK, HPt protein and Ssk1, Skn7 and Rim15-like RRs. In addition, Dual-HKs, unusual hybrid HKs with 2 HK and 2 RR domains, are absent in *A. bisporus* and are limited to various species of basidiomycetes. Differential expression analysis showed no significant up- or down-regulation of the *Agaricus* TCS genes in the conditions/tissue analyzed with the exception of the Skn7-like RR gene (Agabi_varbisH97_2|198669) that is significantly up-regulated on compost compared to cultured mycelia. Furthermore, the pipeline web server BASID2CS (http://bioinformatics.unavarra.es:1000/B2CS/BASID2CS.htm) has been specifically designed for the identification, classification and functional annotation of putative TCS proteins from any predicted proteome of basidiomycetes using a combination of several bioinformatic approaches.

1. Introduction

Two-component systems (TCSs) are signal transduction mechanisms based on phosphotransfer reactions between histidine and aspartate residues (His–Asp phosphorelays) that are used by living cells to sense and respond to extracellular or intracellular events (Perraud et al., 1999; Stock et al., 2000). TCSs are wide-spread across organisms in all three domains of life (Eukaryotes, Bacteria and Archaea) with the exception of higher eukaryotes such as humans and other animals (Grebe and Stock, 1999; Loomis et al., 1997, 1998; West and Stock, 2001; Wolanin et al., 2002). The predominant signaling pathways in eukaryotes are protein phosphorylation cascades involving serine, threonine and tyrosine kinases, and TCS pathways constitute a very small number of all signalling systems (Santos and Shiozaki, 2001; West and Stock, 2001). In fungi, TCSs are required for transmission of an extracellular or intracellular signal after sensing an environmental cue or during normal cell development that results in transcriptional regulation of associated genes (Klein and Tebbets, 2007; Li et al., 2010; Santos and Shiozaki, 2001). Fungal TCS proteins have been often identified as unique upstream components in stress-activated MAPK (mitogen-activated protein kinase) signalling networks, such as the HOG (high-osmolarity glycerol response) MAPK pathway that modulates responses to hyperosmotic shock and other stresses (UV irradiation, oxidative damage and high temperature) (Hohmann et al., 2007). TCS proteins regulate a wide array of distinct processes in fungi: cell growth, differentiation, environmental stress responses, cell wall biosynthesis, biofilm formation, sporulation, drug resistance, dimorphism and virulence (Catlett et al., 2003; Klein and Tebbets, 2007; Kruppa and Calderone, 2006; Li et al., 2010; Santos and Shiozaki, 2001).

In most prokaryotic TCSs there is a physical separation of the input sensory and output regulatory domains with a prototypical phosphotransfer mechanism composed of two signalling proteins, the sensor Histidine Kinase (HK) and the effector Response Regulator (RR) proteins (Santos and Shiozaki, 2001; Wolanin et al., 2002). HKs are often transmembrane proteins that in response to a stimulus autophosphorylate a conserved His residue. In general, fungal TCS signalling cascades involve a multiple His–Asp phosphorelay

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mechanism composed of three functional modules: a hybrid HK carrying both an HK and a C-terminal RR domain in a single polypeptide, a histidine-containing phosphotransfer (HPt) protein, and a RR (Bahn et al., 2006; Li et al., 2010). HPt proteins are commonly small proteins that act as His-phosphorylated intermediates in the transfer of phosphoryl groups from hybrid HKs to RRs (Appleby et al., 1996; Grebe and Stock, 1999; Stock et al., 2000; Wolanin et al., 2002). Generally, fungal genomes encode a single HPt protein and several hybrid HKs and RRs (Bahn et al., 2006; Catlett et al., 2003; Klein and Tebbets, 2007; Lavín et al., 2010; Santos and Shiozaki, 2001). One of the best characterized TCS in fungi is the Sln1 system of *Saccharomyces cerevisiae* (Sln1–Ypd1–Ssk1, Skn7) which modulates the downstream HOG MAPK cascade, where Sln1 is a membrane bound hybrid-HK, Ypd1 a cytoplasmic HPt protein, and Ssk1 and Skn7 are two functionally distinct RRs (Cashin et al., 2006; Fassler and West, 2011; Santos and Shiozaki, 2001).

Basidiomycetes are a large and diverse fungal phylum exhibiting a marked variation in their physiological traits, morphological complexity and lifestyles (Kirk et al., 2001). This phylum contains roughly 30,000 species and comprises three subphyla: *Agaricomycotina* (mushroom-forming fungi), *Pucciniomycotina* (rust fungi) and *Ustilaginomycotina* (smut fungi) (Hibbett et al., 2007; James et al., 2006). Due to their uncertain phylogenetic position within basidiomycetes, species belonging to the class-level taxa Wallemiomycetes (xerophilic mold-like fungi such as *Wallemia sebi*) and Entorrhizomycetes are classified as Basidiomycota *incertae sedis*, not placed in any subphylum (Hibbett et al., 2007; Padamsee et al., 2012). There are 50 complete genome sequences of basidiomycetes currently available at MycoCosm (Grigoriev et al., 2012). The subphylum *Agaricomycotina* encompasses most mushroom-forming fungi including the white button mushroom *Agaricus bisporus*, a secondary decomposer that naturally grows and forms fruiting bodies in grasslands and temperate forests playing an ecologically significant role in the degradation of leaf and needle litter. Moreover, *A. bisporus* is the principal edible mushroom widely cultivated and commercialized all over the world (Sánchez, 2010). Sequencing of the genomic DNA of *A. bisporus* var. *bisporus* (H97) and var. *burnetti* JB137-S8 revealed 30.2- and 32.6-megabase genome assemblies, respectively (Morin et al., 2012). Advancing our knowledge of mushroom-producing fungi holds interest for agriculture, human health, ecology and biotechnological applications (Ohm et al., 2010; Sánchez, 2010).

Up to date, the identification and characterization of fungal TCS proteins have been mainly focused on ascomycetes (Catlett et al., 2003; Hagiwara et al., 2007; Klein and Tebbets, 2007; Kobayashi et al., 2007; Kruppa and Calderone, 2006; Motoyama et al., 2008; Ronning et al., 2005; Santos and Shiozaki, 2001; Schmoll, 2008), and the functions of the TCS proteins in basidiomycetes have remained unexplored except for studies in the ubiquitous environmental fungus *Cryptococcus neoformans* that has seven HKs (Tco1–Tco7), a single HPt protein, and three RRs (Ssk1, Skn7 and Rim15) (Bahn et al., 2006; Bahn, 2008). This human fungal pathogen causes morbid meningioencephalitis and TCS proteins control many aspects of basic and adaptative cellular functions (Bahn et al., 2006; Bahn, 2008). Additionally, other reports present the identification and expression analysis of the hybrid HK Le.nik1 in the Shiitake mushroom *Lentinula edodes* that may be involved in mushroom development and osmotic stress response (Szeto et al., 2008), the genomic analysis of TCS proteins in the brown rot fungus *Postia placenta* (Martinez et al., 2009), and the comparative genomic analysis of TCS proteins in 10 basidiomicetes species (Lavín et al., 2010). The aim of this work was to identify the TCS proteins of the recently sequenced genomes of two *A. bisporus* varieties (Morin et al., 2012), and using the pipeline web server BASID2CS (http://bioinformatics.unavarra.es:1000/B2CS/BASID2CS.htm) extend the analysis to the complete genome sequences of basidiomycetes available at MycoCosm (Grigoriev et al., 2012). We have developed this bioinformatic platform for the identification, classification and functional annotation of putative TCS proteins from any species belonging to this fungal phylum. Finally, all the TCS proteins in *Agaricus* remain to be characterized and the present genomic analysis paves the way for future TCS functional studies in this basidiomycete fungus.

2. Materials and methods

2.1. Identification and analysis of TCS proteins

The genomic sequences and full proteomes of *Agaricus bisporus* var. *bisporus* (H97) and var. *burnetti* JB137-S8 and of the other species of basidiomycetes (Table 1) were obtained from MycoCosm (as of April 2012) (http://jgi.doe.gov/fungi), the integrated fungal genomics resource from the Department of Energy (DOE) Joint Genome Institute (JGI) Genome Portal (Grigoriev et al., 2012).

Genes coding for TCS proteins in the genomes of *Agaricus bisporus* var. *bisporus* (H97) and var. *burnetti* JB137-S8 were identified on the basis of a computational domain analysis of protein sequences similar to that described previously (Lavín et al., 2010). In order to identify the TCS proteins in the predicted proteomes, the HMMER 3.0 software (Finn et al., 2011) was used with different Hidden Markov Models (HMMs) profiles extracted from the Pfam protein families database (Bateman et al., 2004) that target HK (HisKA), receiver (REC) RR, or HPt domains. TCS proteins have a modular architecture with different arrangements of specific conserved domains within proteins (Perraud et al., 1999; Stock et al., 2000; West and Stock, 2001), and domain architecture is particularly useful for the classification of TCS proteins. Functional domains of TCS proteins were identified by search the Conserved Domain Database (CDD) with Reversed Position Specific BLAST (Marchler-Bauer et al., 2003), and identification of orthologous groups was made by phylogenetic tree analysis with the LOFT program (van der Heijden et al., 2007). Multiple sequence alignments of TCS proteins were performed using MUSCLE (Edgar, 2004) and imported into Clustal W (Larkin et al., 2007) to generate phylogenetic trees that were visualized in LOFT (van der Heijden et al., 2007), a tool that automatically identify orthologous groups. Default parameters were used. Prediction of transmembrane spanning regions in HKs was carried out using TMHMM v 2.0 (http://www.cbs.dtu.dk/services/TMHMM-2.0/) (Krogh et al., 2001).

Furthermore, genes coding for TCS proteins in the genomes of the other species of basidiomycetes were identified, classified and functionally annotated through the pipeline web server BASID2CS (see below) following the bioinformatic protocol used in *Agaricus* genomes.

2.2. The pipeline web server BASID2CS

With the increasing number of whole genome sequences and predicted proteomes of basidiomycetes already available, we consider that a web-based tool to identify TCS proteins for comparative and functional studies could be very valuable. The BASID2CS pipeline is specifically designed and implemented for the bioinformatic screening and extraction of all the putative TCS proteins from each predicted proteome of basidiomycetes in a single step. Additionally, BASID2CS integrates the previously described bioinformatic tools providing a fast and efficient pipeline especially suited to explore the properties of TCS proteins, and classify them. A major advantage of BASID2CS is that allows custom analysis with user-defined cut-off values and extraction of TCS protein sequence data. The BASID2CS is freely available on the web at http://bioinformatics.unavarra.es:1000/B2CS/BASID2CS.htm and is compatible with all major