C. R. Biologies 337 (2014) 29–43

Contents lists available at ScienceDirect

Comptes Rendus Biologies

www.sciencedirect.com

Microbiology: bacteriology, mycology, parasitology, virology/Microbiologie : bactériologie, mycologie, parasitologie, virologie

Characterization of the *aap1* gene of *Agaricus bisporus*, a homolog of the yeast YAP1

Caractérisation du gène aap1 *d'*Agaricus bisporus*, un homologue du gène YAP1 de la levure*

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ARTICLE INFO

Article history:
Received 27 August 2013
Accepted after revision 25 October 2013
Available online 19 December 2013

Keywords:
bZIP
AP-1-like transcription factor
Yap transcription factor
Temperature adaptation
Mushroom

ABSTRACT

The structure, homologies, polymorphism and expression profiles of a new gene, *aap1*, have been studied for precisely characterizing it and defining its putative involvement in thermo-tolerance of both vegetative mycelium growth and sporophore differentiation. Sequence polymorphism was analyzed in 3 homokaryons of *A. bisporus* and 24 strains having different abilities for mycelial growth at temperatures above 30 °C and for producing mature fruiting bodies at 25 °C. The level of gene expression was measured by real-time PCR both in vegetative mycelium after transfer from 25 to 32 °C and in primordia and fruiting bodies produced during cultures at 17 or 25 °C. The results indicated that *aap1* gene belong to a new subfamily of the yeast *YAP1* homologs. It is not a dominant contributor to the thermo-tolerance of *A. bisporus*, but the protein it encodes may be involved as an overall stress resistance transcription factor. The way Aap1 senses redox level differs from that of AP-1-like transcription factor Yap1.

Mots clés :
bZIP
Facteur de transcription de type AP-1
Facteur de transcription Yap
Adaptation à la température
Champignon de couche

RÉSUMÉ

Structure, homologies, polymorphisme et profil d'expression d'un nouveau gène, *aap1*, sont étudiés pour en réaliser une caractérisation précise et établir son implication potentielle tant dans la thermo-tolérance de la croissance mycélienne que dans la différenciation du sporophore. Le polymorphisme des séquences est analysé chez des souches ayant différentes capacités de croissance mycélienne à des températures supérieures à 30 °C et pour produire des fructifications matures à 25 °C. Le niveau d'expression des gènes est mesuré par PCR en temps réel, tant dans le mycélium après transfert de 25 à 32 °C que dans les primordia et les corps fructifères produits lors des cultures à 17 et 25 °C. Les résultats montrent que le gène *aap1* appartient à une nouvelle sous-famille des homologues du gène *YAP1* de levure. Il n'est pas un contributeur dominant pour la thermo-tolérance d'*A. bisporus*, mais la protéine qu'il code peut être impliquée comme facteur de transcription de résistance générale au stress.

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1631-0691/$ – see front matter
http://dx.doi.org/10.1016/j.crvi.2013.10.010

1. Introduction

Agaricus bisporus (Lange) Imbach, the button mushroom, is a leaf-litter rot fungus. Of the major cultivated mushrooms, it has until recently been the least well known from nature. Since 1990, numerous isolates have been collected throughout the world at different sites on different kinds of decomposing lignocellulosic materials, and three varieties having different life cycles have been identified [1,2]. For many years, the button mushroom has been grown in caves at 15–16 °C. Since the 1980s, climatic chambers replace caves, and cultivation conditions are standardized, with a 16–18 °C temperature recommended during the cropping period, based on the requirement of the cultivated strains. Thermo-tolerance is an interesting trait to be studied, both for the cultivation of the mushroom out of temperate countries, and for the understanding of the distribution of wild populations under various climates. Usually, a temperature over 32 °C stops the growth of the vegetative mycelium. In a recent work, 91 isolates from various geographic areas were screened for their ability to fruit at higher temperatures (FHT^+ strains fruiting at 25 °C) than the commercial cultivars. The mushroom variety discriminated for this trait. *Agaricus bisporus* var. *eurotetrasporus* was unable to develop any sporophore whilst *A. bisporus* var. *burnettii* was well adapted to fruit both at 17 and 25 °C, suggesting this phenotypic plasticity is a fixed trait in this variety. In the more common *A. bisporus* var. *bisporus*, the ability to fruit at 25 °C was observed in some strains, but it correlated neither with climate/microclimate nor with habitat of the strains, and yields were always lower than at 17 °C [3]. However, we do not know the temperature at which the ancestor of the species fruited, neither the order in which the three varieties were separated.

To date, information on thermo-tolerance mechanisms of *A. bisporus* is scarce. Another cultivated mushroom, *Flammulina velutipes* (Curtis) Singer is generally regarded as a fungus that requires a low temperature (< 15 °C) for the induction or production of fruiting bodies, as compared with vegetative growth, which has an optimum temperature of 22 to 26 °C [4,5]. Fultz [6] analyzed progenies of five crosses between strains with high and low fruiting temperature indicating that a minimum of two genes appeared to control the requirement for fruiting at > 15 °C. Mutants able to fruit at 20 °C and, for most of them, to grow at 33 °C were recently obtained by ultraviolet irradiation mutagenesis and combined mutagenesis [7]. In filamentous Ascomycota fungi, over-expression of a heat shock proteins (HSP70) enhances thermo-tolerance of *Trichoderma harzianum* [8]. Heat shock factor proteins (HSFs) are transcriptional regulators of genes that encode different types of stress proteins as HSPs. In *Coniothyrium minitans*, HSF1 over-expression enhances tolerance to heat stress [9].

For *A. bisporus*, Chen [10] obtained few thermo-tolerance-related gene fragments with DD-RT-PCR to analyze gene expression in the vegetative mycelium of one *A. bisporus* strain (02) under normal and higher-temperature cultivation. They identified 3 genes of thermo-tolerance: *028-1*, *023-11A* and *023-11B* [10–14]. The full-length cDNA sequence of one gene (*028-1* GenBank accession number DQ235473) had been obtained [13,15]. It has a size of 1.37 kb, and no obvious homological sequence was found in GenBank. The Chinese group constructed a binary expression vector of *028-1* of *A. bisporus* and transferred the gene into the non-thermo-tolerant strain 8213 of *A. bisporus* by *Agrobacterium*-mediated transformation. Eight out of ten of transgenic strains were able to grow at 34 °C as well as the thermo-tolerant strain from which *028-1* had been identified, whereas the wild strain did not [14]. However both in the thermo-tolerant parent and the transformants, the mycelial growth rate was strongly affected, with mycelial diameters after 22 days at 34 °C being 15 to 25% that at 28 °C. Despite the potential interest of this gene and its regulation, no other article than those published by Chen et al. was available. Therefore we proposed that an improvement of its annotation, a study of its polymorphism and level of expression in different strains should allow characterizing *028-1* and determine accurately the role of this gene in thermo-tolerance of *A. bisporus*.

The aim of the present work was then to progress in the knowledge of *028-1* gene and its involvement in the thermo-tolerance of some *A. bisporus* strains. The level of expression of *028-1* in the vegetative mycelium during a heat treatment was analyzed by Q-PCR on several strains. We identified and annotated *028-1* in the genome of the homokaryon of the hybrid HU1 (H97) that had been used for the whole genome sequencing of *A. bisporus* var. *bisporus* (http://genome.jgi-psf.org/Agabi_varbisH97_2/Agabi_varbisH97_2.home. html), but this hybrid is not thermo-tolerant. We established that *028-1* is a homolog of the *YAP1* gene involved in oxidative stress response in yeast.

Yap1p is an AP-1-like transcription factor. AP-1 is an activator protein that links to the promoter of the human metallothionein gene and the simian virus 40 (SV40) [16]. Yap1p is one of the 15 basic-leucine Zipper (bZIP) transcription factors implicated in various forms of stress response in yeasts. These are clustered in 7 families, 4 conserved in metazoans (Atf2, Atf6, CREB1 and Jun) and 3 only found in yeast (Yap, Met4 and Met28). The YAP (yeast activator protein) family includes eight members (Yap1-Yap8) [17–19]. Three of these members (Yap1, Yap2 and Yap8) contain Yap1 redox domain (IPR023167). *A. bisporus* 028-1 protein also contains this domain but differs from Yap1P in his sensing capability. That is why we propose to name *aap1* the gene *028-1*.

Data on *028-1* polymorphism and level of expressions in primordia and sporophores obtained at two temperatures in different strains are presented and discussed on the light of its putative role in thermo-tolerance of *A. bisporus*.

2. Materials and methods

2.1. *Strains of* Agaricus bisporus

The strains used in the different parts of the work are presented in Table 1. They all are maintained in Collection Germplasm of *Agaricus* at Bordeaux (CGAB) INRA, France.