Induction and Transcriptional Regulation of Laccases in Fungi

Alessandra Piscitelli, Paola Giardina, Vincenzo Lettera, Cinzia Pezzella, Giovanni Sannia and Vincenza Faraco*

University of Naples “Federico II”, Dipartimento di Chimica Organica e Biochimica, Complesso Universitario Monte S. Angelo, via Cintia 4, 80126 Napoli, Italy

Abstract: Fungal laccases are phenol oxidases widely studied for their use in several industrial applications, including pulp bleaching in paper industry, dye decolourisation, detoxification of environmental pollutants and valorization of wastes and wastewaters. The main difficulty in using these enzymes at industrial scale ensues from their production costs. Elucidation of the components and the mechanisms involved in regulation of laccase gene expression is crucial for increasing the productivity of native laccases in fungi. Laccase gene transcription is regulated by metal ions, various aromatic compounds related to lignin or lignin derivatives, nitrogen and carbon sources. In this manuscript, most of the published results on fungal laccase induction, as well as analyses of both the sequences and putative functions of laccase gene promoters are reviewed. Analyses of promoter sequences allow defining a correlation between the observed regulatory effects on laccase gene transcription and the presence of specific responsive elements, and postulating, in some cases, a mechanism for their functioning. Only few reports have investigated the molecular mechanisms underlying laccase regulation by different stimuli. The reported analyses suggest the existence of a complex picture of laccase regulation phenomena acting through a variety of cis acting elements. However, the general mechanisms for laccase transcriptional regulation are far from being unravelled yet.

1. LACCASE INDUCTION

Fungal laccases are phenol oxidases belonging to the group of multi-copper enzymes that catalyze the oxidation of a great variety of phenolic compounds and aromatic amines using molecular oxygen as electron acceptor [1]. Almost all the fungi examined secrete more than one laccase isozyme [1]. Due to their action over a broad range of substrates, fungal laccases have been widely studied for their use in several industrial applications, including pulp bleaching in paper industry, dye decolourisation, detoxification of environmental pollutants and valorization of wastes and wastewaters [2-4]. Research on regulation of laccase gene expression may be very useful for increasing the productivity of native laccases in fungi and also for unravelling the physiological role of the different isoforms produced by the same organism. Synthesis and secretion of laccases are strictly influenced by nutrient levels, culture conditions, developmental stage as well as the addition of a wide range of inducers to cultural media, with variations among both different fungal species and different isoforms in a same strain. The effect of these factors at the level of laccase gene transcription has been demonstrated in many fungal species. Laccase gene transcription is often regulated by metal ions [5, 6], various aromatic compounds related to lignin or lignin derivatives [7], nutrient nitrogen [5] and carbon [8]. In most of the reported examples, laccase expression is regulated by an array of factors, acting in a synergistic or antagonistic way [9-12]. Physiological mechanisms occurring during mycelia development can also modulate the relative expression levels of laccase isoenzymes. Some isoforms have been observed during the lag and exponential phases of fungal fermentation and should be involved in substrate degradation, whilst other isoforms have been detected in the stationary phase and should be related to mushroom morphogenesis and pigmentation processes [13, 14].

1.1. Laccase Induction by Metals

Regulation of laccase expression by metals is widespread in fungi. In *Trametes versicolor* 290, the highest laccase activity value (2500 U L⁻¹) was achieved in cultures grown in the presence of 400 μM Cu²⁺, corresponding to a 18-fold higher level than in the absence of copper [5]. Galhaup and Haltrich [15] have studied the stimulatory effects of copper on the laccase production by different *Trametes spp*. The laccase activity of *Trametes versicolor* MB 52, *Trametes versicolor* MB 54, *Trametes suaveolens* MB 51 was induced by 1 mM Cu²⁺, reaching values of about 9000, 10000, and 7000 U L⁻¹, respectively, while none of these strains produced laccase activity value (2500 U L⁻¹) without additional Cu²⁺ [15]. Lorenzo et al. [16] studied the effect of copper on laccase production of *Trametes versicolor* (CBS100.29). The highest values were obtained by cultures supplemented with 3.5 mM Cu²⁺, showing an increase of 11-fold and maximum values around 8000 U L⁻¹. More recently, a higher laccase production induced by
copper was observed by the *Trametes* sp. 48424. In this case, the highest laccase activity in the presence of 1 mM Cu$_{2}^{+}$ was 15273 U L$^{-1}$ [17].

Even in *Pleurotus* spp. the induction of laccase by copper has been widely documented. Giardina et al. [18] obtained a laccase production of 30000 U L$^{-1}$, growing *Pleurotus ostreatus* (ATCC MYA-2306) in nutrient-rich medium, with addition of 150 µM CuSO$_4$, whereas laccase production resulted between 0.5 and 4 U L$^{-1}$ in the presence of copper traces. More recently, a laccase production of 70000 U L$^{-1}$ was observed when this fungus was grown in semi-synthetic growth media formulated with agro-industrial by-products and supplemented with 150 µM CuSO$_4$ (unpublished data).

In all the analysed fungi (*T. versicolor*, *Ceriporiopsis subvermispora* [19], *P. ostreatus* [20, 11], *P. sajor-caju* [8], *Coriolopsis rigida* [3] and *Trametes pubescens* [6]), regulation of laccase production by copper has been demonstrated to occur at transcription level. In *P. ostreatus* (ATCC MYA-2306), Cu$_{2}^{+}$ has a marked effect on the induction of the examined isoenzymes POXC, POXA3 and POXA1b. The effect of Cu$_{2}^{+}$ on laccase synthesis can be explained not only at transcriptional level. The results of Palmieri et al. [21] showed that Cu$_{2}^{+}$ concentration of 1 mM decreases the extracellular proteolytic activity, thus impairing laccase degradation.

Ag$^{+}$, and Mn$^{2+}$ ions have also been reported as modulators of laccase transcription [8,10]. It is worth noting that the same metal can exert opposite effects in different fungal species [10]. As a matter of fact, Mn$^{2+}$ acts as an inducer of laccase transcription in *P. sajor-caju* [8], *Clitocybula dusenii* and *Nematoloma frowardii* [22], but it was shown to inhibit laccase expression in *C. subvermispora* [10].

### 1.2. Laccase Induction by Aromatic Compounds

Phenolic and aromatic compounds structurally related to lignin or lignin derivatives are routinely added to fungal cultures to increase laccase production [23, 24]. Laccase induction by phenolic substances may represent a response developed by fungi against toxic aromatic compounds. By catalyzing their polymerization, laccases play a defensive role, reducing the oxidative stress caused by oxygen radicals originating from the reaction of these molecules [25]. An overview of the inductive responses by several fungi to different aromatic compounds is reported in Table 1 [26-47]. Laccase induction varies with the organism and seems to be specific to certain aromatic compounds.

The effect of veratryl alcohol on laccase production varies also with the composition of basal media used [48], enhancing laccase production from 2- to 200-fold. Guaiacol supplementation (1 mM) enhances laccase production from 2- to 232-fold in different fungi, giving maximum stimulatory effect in *Phlebia spp.* followed by *P. ostreatus* [24]. However, there is also evidence of suppression of laccase production by guaiacol in *Pycnoporus cinnabarinus* [41].

Among the 15 different phenolic compounds tested in *P. pulmonarius*, ferulic acid and vanillin produce the highest levels of laccase activity, inducing the expression of lcc3 isoenzyme, not detectable in basal condition [23]. Screening of exogenous aromatic compounds for their effect on *P. ostreatus* laccase induction has shown ABTS (0.5-1 mM) as the most significant inducer, enhancing laccase activity production up to five-fold. 2,5-Xyldine, guaiacol and ferulic acid (1 mM) also increase laccase activity from two to three-fold, whereas veratryl alcohol does not stimulate enzyme production [24]. Moreover, the presence of ferulic acid (2 mM) in liquid culture of *P. ostreatus* (ATCC MYA-2306) also produces a different laccase isoenzyme pattern [20].

Laccase induction has also been demonstrated in the presence of dyes, the induction level being highly sensitive to small differences in their chemical structures [49].

Besides molecular structures and concentration, the inductive effect of phenolic compounds depends also on the time of their addition to the cultures [50]. In *P. pulmonarius* and *Botryosphaeria rhodina* cultures, the induction seems to be more efficient when the inducer was added to the cultures at inoculum time [23, 51], whereas in *P. ostreatus*, *Rigidoporus lignosus* and *Trametes modesta* medium conditioning is usually performed after 2-5 days from inoculation in order to avoid growth inhibition [52, 53]. High concentrations (>1 mM) of exogenous aromatic compounds in culture medium can negatively affect the fungal growth if the mycelium physiological state is not yet adapted to the changes of growth conditions or if it is compromised by environmental or mechanical stresses (preinoculum homogenization or sub-culturing from agar plate to liquid culture).

The induction of aromatic compounds has been found to occur at transcriptional level, differing not only among the various fungi analyzed, but also among the different isoenzymes of the same organism. In *T. versicolor*, both 1-hydroxybenzotriazole and 2,5-xyldine effectively activate lcc transcription, whereas no induction is observed in the presence of either veratric acid or ferulic acid [5]. Transcription of one laccase gene, lcc1, from *T. villosa* is induced by the addition of 2,5-xyldine, but a second gene is constitutively expressed under the tested conditions [54]. In the ligninolytic basidiomycete *Trametes* sp. 162, nine structural closely related aromatic compounds appear to have different effects on laccase gene expression. The three laccase genes of this fungus are differentially expressed in response to these compounds, with specific induction patterns being observed for each molecule tested [7].

In the basidiomycete *Trametes* sp. AH28-2, where three novel laccase genes, *lacA*, *lacB*, and *lacC*, have been recently identified, it has been reported that different aromatic compounds can selectively induce the production of distinct laccase isozymes, with o-toluidine inducing the expression of LacA and 3,5-dihydroxytoluene mainly stimulating the production of LacB. Analysis by competitive Real Time Polymerase Chain Reaction showed that the accumulation of laccase RNA transcripts is accompanied by the increase of corresponding enzyme activity in cultures [55].

In *Volvariella volvacea*, lcc1 transcript titres are differentially tuned by the addition of various aromatic compounds, whereas lcc4 transcription is not affected by these molecules [56].
| References                | ABTS | Caffeic acid / caffein | Catechol | Dimethylphenol | Ferulic acid | Gallic acid | Guaiacol | Hydroquinone | Hydroxybenzoic acid | Syringic acid | Vanillin | Vanillic acid | Veratric acid | Veratryl alcohol | 2,5-xylidine |
|--------------------------|------|------------------------|----------|----------------|--------------|-------------|-----------|-------------|---------------------|---------------|----------|---------------|---------------|------------------|--------------|
| Ceriporiopsis subvermispora | [26] |                        |          |                |              |             |           |             |                     |               |          |               |               |                  |              |
| Cerrena unicolor          | [27] | +                      | +        | -              |              | -           | -         | +           | +                   |              | -        |               |               |                  |              |
| Clitocybula dusenii       | [22] |                        |          |                |              |             |           |             |                     |               |          |               |               |                  |              |
| Coprinus friesian        | [28] |                        |          |                |              |             |           |             |                     |               |          |               |               |                  |              |
| Coriolopsis rigida       | [3]  | -                      |          |                |              |             |           |             |                     |               |          |               |               |                  |              |
| Daedalea flavida         | [29] |                        |          | +              | +            |             |           |             |                     |               |          |               |               |                  |              |
| Dichomitus squalens      | [31] |                        |          |                |              |             |           |             |                     |               |          |               |               |                  |              |
| Ganoderma lucidum        | [27] |                        | -        | -              |              |             |           |             |                     |               |          |               |               |                  |              |
| Lentinula edodes         | [32] |                        | -        |                |              | -           | -         |             |                     |               |          |               |               |                  |              |
| Lentinus strigosus       | [33] |                        | +        | +              |              |             |           |             |                     |               |          |               |               |                  |              |
| Mycena galopus           | [34] |                        |          |                |              | -           | -         | +           |                     |               |          |               |               |                  |              |
| Marasmius spp.           | [35] |                        | +        | +              |              |             |           |             |                     |               |          |               |               |                  |              |
| Nematoloma frowardii     | [22] |                        |          |                |              |             |           |             |                     |               |          |               |               |                  |              |
| Phanerochaete chrysosporium | [37] |                        |          | +              | +            |             |           |             |                     |               |          |               |               |                  |              |
| Phlebia spp.             | [30] |                        |          |                |              | +           |           |             |                     |               |          |               |               |                  |              |
| Pleurotus eryngii        | [40] |                        |          |                |              |             |           |             |                     |               |          |               |               |                  |              |
| Pleurotus ostreatus      | [24] | +                      |          | +              | +            |             |           |             |                     |               |          |               |               |                  |              |
| Pleurotus pulmonarius    | [23] | +                      |          | +              | +            |             |           |             |                     |               |          |               |               |                  |              |
| Pleurotus sajor-caju     | [8]  | +                      |          |                | +            |             |           |             |                     |               |          |               |               |                  |              |
laccase expression induced in presence of the corresponding compound.
- laccase expression inhibited or not induced in presence of the corresponding compound.
*Contrasting results are reported for this compound in [5] and [27].
where nothing is shown, the corresponding compound has not been tested yet for that strain.

1.3. Laccase Regulation by Nitrogen and Carbon Sources

Laccase activity has also been shown to be dependent on the concentration and nature of carbon and nitrogen sources as well as on their ratio. Nitrogen source plays a key role in laccase production, with effects depending on its nature and concentration in culture media [41]. Change in laccase activity in response to nitrogen concentration is a controversial issue, since examples of activity increases have been described under both limiting and non-limiting conditions. Generally, inorganic nitrogen sources lead to low levels of laccase with sufficient biomass production, while organic nitrogen sources give high laccase yields with good fungal growth. Yeast extract is one of the best nitrogen sources that increases the yield of laccase enzymes [31]. The enzyme yield is also increased by supplementation of the medium with an additional nitrogen source like the amino acid L-asparagine [57].

Laccase induction at transcriptional level has been reported to occur under non-limiting nitrogen levels in the ligninolytic basidiomycete I62 (CECT 20197), where lcc1 and lcc2 transcript levels increased 100-fold in comparison with limiting nitrogen conditions [58]. Expression of lcc1 in *Trametes trogii* is preferentially induced by organic nitrogen sources compared with inorganic ones [59]. Individual laccase genes in *P. sajor-caju* are differentially induced at the transcriptional level by nitrogen sources, with a positive regulation by nitrogen occurring for lac2 and lac4, and no effect being detected for lac1 and lac3 [8].

As far as carbon sources are concerned, it has been demonstrated that supplementation of substrates, like glucose that are readily utilizable and efficiently metabolized by the microorganism, results in high levels of laccase activity [15]. Studies of laccase optimization in *P. ostreatus* IMI 395545 submerged cultures [12] have shown that glucose leads to the highest production of laccase compared to other carbon sources. As a matter of fact, fivefold increase of the laccase activity has been reported to occur under glucose concentration up to 20 g L⁻¹.

In some fungi, laccase expression has been found to be subjected to catabolite repression: this mechanism is thought to be an energy-saving response. High carbon levels (around 15 g L⁻¹) inhibit laccase transcription in basidiomycete I62 [58], *T. pubescens* [6], and *Trametes sp.* AH28-2 [55].

2. LACCASE PROMOTERS SEQUENCE ANALYSIS

*In silico* inspection of several laccase promoter sequences highlights the presence of many different responsive
elements differentially distributed along the promoter sequences. Promoter analyses allowed a correlation between the observed regulatory effects on laccase gene transcription and the presence of specific responsive elements, and to postulate in some cases, a mechanism for their functioning. Differences in copy number, location or orientation of the putative response elements determine a complex picture of laccase regulation phenomena.

The promoter region of the gene coding for the major laccase isoenzyme LAP2 from *T. pubescens*, extending about 1420 bp upstream of the start codon ATG, has been analyzed by Galhaup et al. (2002) [6]. This upstream region contains the usual promoter elements i.e. TATA box and seven CAAT motifs, along with two MREs (Metal Responsive Element), four CreA consensus sequences, 27 potential HSEs (Heat shock elements) [60] and a “general” STRE (Stress responsive element). Expression of LAP2 was found to be highly induced by copper and other heavy metal ions but repressed by glucose. Metal effect can be related to the presence of multiple MREs, causing a high level of induction [61]. Cis-acting MREs have been discovered in multiple copies in the *Saccharomyces cerevisiae* metallothionein promoter where they are essential for efficient metal-inducible transcription [62]. The presence of putative CreA-binding sites in the *lap2* promoter region suggests the existence of a carbon catabolite represor similar to the CreA isolated in *A. nidulans* [63]. HSE and STRE might be involved in stress-regulated *lap2* gene expression caused, for example, by high concentrations of copper. Interestingly, neither xenobiotic response elements (XREs) nor antioxidant response elements (ARE) could be detected in the *lap* upstream region in accordance with the lack of transcriptional induction observed in the presence of aromatic substances [6].

Four putative MRE consensus sequences have been also found in the promoter region (~1kb long) of the copper inducible LAC2 laccase from *Gaeumannomyces graminis*, two of which are inverted and 51bp apart. In addition, this promoter contains two putative ACE1 (activation of *cup1* expression protein) binding sites situated between the TATA box and ATG codon [64]. First described in *S. cerevisiae*, ACE binding site is the recognition site for the ACE1 copper-responsive transcription factor, which binds its target sequence in a copper-loaded conformation, activating the transcription of several genes [65]. An ACE binding sequence has also been found in the promoter of the copper induced laccase *lc5* from *C. subvermispora* [19].

Soden and Dobson [8] have performed an in-depth study on the promoter regions of four laccase genes (*lac1*, *lac2*, *lac3* and *lac4*, extending 724bp, 214bp, 840bp and 1740bp upstream of the start codons, respectively) from *P. sapor-caju* displaying differential regulation in response to different stimuli [66]. A number of putative response elements including MRE, XRE, ARE (Antioxidant response element) and HSE have shown to be differentially distributed in the analysed promoter regions. The presence of consensus sequences putatively involved in nitrogen and carbon regulation such as CreA, Mig and NIT2 [67] binding sites, may explain the regulation by nutrient carbon and nitrogen sources, detected for some *P. sapor-caju* laccase genes [8], mediated by Mig1p or NIT2-like proteins. A single MRE has been identified in the promoter region of the strongly copper-induced *lac4* gene, whilst sequences with perfect homology with XRE have been detected in *lac1* and *lac4* promoters. Interestingly, laccase transcriptional analysis indicated that both *lac1* and *lac4* are regulated by the addition of 2,5-xylidine and ferulic acid, while *lac2* and *lac3*, both of which appear to lack XRE, are constitutively expressed under these conditions.

The differential expression of three laccase genes (*lacA*, *lacB* and *lacC*) from *Trametes* sp. AH28-2, has been investigated [55]. An inferred TATA box and several putative CAAT, MRE, XRE and CreA consensus sequences have been identified in the *lacA*, *lacB* and *lacC* promoter regions (1881bp, 993bp and 1703bp long, respectively). Differences in copy number and distribution of XREs among the three genes (seven XREs in *lacA* and only two in *lacB* and *lacC*) are supposed to be responsible for their different responses to aromatic compounds: *lacA* is induced by all tested aromatic compounds, *lacB* is induced mainly by guaiacol and 3,5-dihydroxytoluene, whilst *lacC* transcript levels are not detectable in the presence of these compounds. The overlapping of one XRE element with the TATA box in the *lacC* promoter is probably responsible for the absence of induction of this gene by aromatic compounds.

In addition to the above reported basidiomycetes, the presence of putative MREs has also been discovered in laccase promoter genes from the aquatic fungus *Myriocionium* sp. UHH 1-13-18 [68], and in the ascomycete *Hortea acidophila* [69] implying their potential role in copper induction of laccase expression.

The 1718 bp 5'-upstream region of a new laccase gene from *Trametes* sp. 48424 has been inspected by Fan and colleagues and compared with that of other known laccase genes from *Trametes* species [17]. This analysis highlighted the presence of new putative regulatory sites in the *lac48424-1* promoter region. Two putative iron responsive elements and three putative cAMP-responsive elements have been mapped in the upstream region, suggesting a role for cAMP in transcriptional regulation of laccase genes, as already proved for lignin and manganese dependent peroxidases in *Phanerochaete chrysosporium* [70]. Finally, the potential contribution of the Skn7 response regulator, a factor involved in the oxidative stress response in *S. cerevisiae* [71], in controlling laccase expression, has been hypothesized by the presence of two putative Skn7 regulatory elements in the *lac48424-1* promoter region.

Twelve putative laccase genes have been identified in the recently sequenced *P. ostreatus* genome (http://www.jgi.doe.gov/sequencing/why/50009.html), one of which is annotated as a ferroxidase-like. The sequence similarity and the intron–exon structure, along with the degree of identity among the corresponding protein sequences, allowed us to individuate two “laccase subfamilies”, while *poxa3* gene is evolutionary distant from both subfamilies [72]. The promoter regions of all *P. ostreatus* laccase genes, extending 500bp upstream of the start codon, have been analysed, revealing very little identity degree among them. *In silico* analysis allowed the identification of several putative response elements,
differentially distributed along the promoter sequences (Fig. 1). It is worth noting that the promoters of the most abundantly produced proteins (POXC and POXA3) display the highest number of signals belonging to different classes. Furthermore some characteristics are peculiar to each subfamily, i.e. the high number of ARE, and the absence of XRE and C and N nutrient responsive element in the $poxa1b$ sub-family.

3. FUNCTION OF LACCASE PROMOTERS

Despite the extensive data available by in silico analysis of many laccase promoters, only a few reports go through the molecular mechanisms underlying laccase regulation by different stimuli.

Some reports have been devoted to the comprehension of molecular aspects leading to laccase induction by copper. In $P. ostreatus$ (ATCC MYA-2306), the formation of protein complexes on specific MREs identified in the promoter regions of the two copper induced laccase genes $poxc$ and $poxa1b$ [20], was verified by electromobility shift assays (EMSA) and foot-printing analysis [11]. EMSAs allowed the evaluation of the ability of the identified MREs to bind proteins from fungal extracts; all putative MREs in the $poxc$ and $poxa1b$ promoters (four MREs in each promoter region) are recognized by fungal proteins, except for one MRE in $poxc$ promoter region, that is located downstream of the transcription-initiation site. Interestingly, the formation of complexes between the identified MREs and fungal proteins was found to occur in the absence of metal ions, suggesting the involvement of a 25 kDa negative-acting regulatory factor able to repress gene expression when bound to laccase promoter. Moreover, the role of specific nucleotides of the identified MREs in complex formation has been analysed, leading to the determination of an affinity scale of the tested MREs and to the definition of the optimal binding sequence. Results of UV-cross-linking analyses indicated that the highest affinity sequence binds also a 30 kDa factor. However, when a GC-rich region is located adjacent to the MRE site, this interaction is inhibited by the binding of a 15 kDa protein. A similar behaviour was also found in some metallothionein promoters where a regulatory role of Sp1, binding to this GC-rich region, in MRE-mediated transcriptional activation has been demonstrated [73].
An ACE1-like factor has been isolated in *P. chrysosporium* (Pc-ACE1-like) trough complementation of a *S. cerevisiae* ace1Δ strain [74]. This factor was found to bind, in the presence of cuprous ions, its target ACE sequence in the promoter region of *mco1* gene, inducing gene expression. Using an *in vitro* transcripational activation system, it was also demonstrated that Pc-ACE1-like is able to activate the transcription of a promoter-reporter construct in the presence of only one ACE element [75]. Intriguingly, copper supplementation to *P. chrysosporium* cultures also caused an increase of *mco2* mRNA levels, although *mco2* promoter regions lacks ACE elements. This effect may be ascribed to a different mechanism, possibly involving the generation of reactive oxygen species [75].

An ACE1-like factor was also isolated from *C. subvermispora* (Cs-ACE1-like). Specific binding of this factor to its cognate ACE sequence in the promoter of the copper induced *lcs* laccase was demonstrated by EMSA assays. This factor exhibited structural differences along with a lower complementation efficiency of the above mentioned *S. cerevisiae* strain, in comparison with its orthologue from *P. chrysosporium* [19]. In particular, the Cs-ACE1-like factor is considerable smaller than Pc-ACE1, lacking the second half of the CRD (Copper regulatory domain) present in ACE1 from *S. cerevisiae* and its orthologues. Although binding capacities of Cs-ACE1 to ACE elements is unaltered, its structural features may be responsible of the rather weak complementation of the *S. cerevisiae* ace1Δ strain.

Mechanisms driving copper regulation have been also analysed in the pathogenic fungus *C. neoformans*, where LAC1 is a copper regulated laccase acting as a virulence factor [76]. In this fungus, copper induction of laccase expression was found to require the copper-responsive factor-encoding gene CUF1, since its disruption abolishes metal effect. The cryptococcal Cuf1 shares peptide sequence homology and similar biological functions to the orthologues. Although binding capacities of Cs-ACE1 to ACE elements is unaltered, its structural features may be responsible of the rather weak complementation of the *S. cerevisiae* ace1Δ strain.

In plant pathogens, ambient pH may affect the expression of putative virulence factors, including laccases. Responses to pH variations occur via a conserved signalling cascade whose terminal component is the zinc-finger transcription factor PacC/Rimp1p [80]. PacC is a key component acting both as a transcriptional activator of alkaline-expressed genes and repressing acid-induced genes. The construction of two *pacC* mutants mimicking acidity or alkaline phenotypes allowed to infer the involvement of PacC in modulating laccase transcription in the plant pathogenic fungus *F. oxysporum*. As a fact, expression of *lcc3* occurs in acidic conditions and is down regulated by PacC, while expression of *lcc5* occurs only at pH 6 and is up regulated by the same factor. The presence of PacC binding consensus sites in *lcc3* and *lcc5* promoter regions further supports these data.

Oxidative stress response has been investigated in the fungus *Magnaporthe oryzae*, the causal agent of rice blast disease. Deletion of *Motif1* gene, an orthologue of *Schizosaccharomyces pombe* ATF/CREB involved in oxidative stress response, caused retarded vegetative growth of mycelium together with reduced transcriptional level of laccases and peroxidases. As a fact, the basic leucine zipper transcription factor Motif1 was found to be necessary for full virulence of the fungus by regulating the transcription of laccases and peroxidases to impair reactive oxygen species-mediated plant defence [81].

In conclusion, the reported analyses, seen as a whole, suggest a complex picture of laccase regulation phenomena whose precise molecular mechanisms through a variety of putative *cis* acting elements is only partially known. Further studies may provide additional clues to unravel the mechanism underlying laccase transcriptional regulation and to verify its general occurrence in different species.

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