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ORIGINAL PAPER

Bacterial laccases

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Abstract Laccases (benzenediol oxygen oxidoreductases, EC 1.10.3.2) are polyphenol oxidases (PPO) that catalyze the oxidation of various substituted phenolic compounds by using molecular oxygen as the electron acceptor. The ability of laccases to act on a wide range of substrates makes them highly useful biocatalysts for various biotechnological applications. To date, laccases have mostly been isolated and characterized from plants and fungi, and only fungal laccases are used currently in biotechnological applications. In contrast, little is known about bacterial laccases, although recent rapid progress in the whole genome analysis suggests that the enzymes are widespread in bacteria. Since bacterial genetic tools and biotechnological processes are well established, so developing bacterial laccases would be significantly important. This review summarizes the distribution of laccases among bacteria, their functions, comparison with fungal laccases and their applications.

Keywords Bacterial laccases · Occurrence · Comparison with fungal laccases · Applications

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Introduction

Laccases are the most numerous members of the multi-copper protein family, which also includes tyrosinases, monoxygenases, and dioxygenases. Phylogenetically, these enzymes have developed from small sized prokaryotic azurins to eukaryotic plasma proteins ceruloplasmin (Claus 2003). They contain four histidine-rich copper-binding domains, which coordinate the types 1–3 copper atoms that differ in their environment and spectroscopic properties (Messerschmidt and Huber 1990). The bacterial azurins, e.g., crystallized rusticyanin from *Thiobacillus ferrooxidans*, which contain only type 1 copper, can be regarded as the precursor protein of laccases (Hough et al. 2001). Laccases are the model enzymes for multi-copper oxidases and participate in (1) cross-linking of monomers, (2) degradation of polymers, and (3) ring cleavage of aromatic compounds (Kawai et al. 1988). For catalyzing the oxidation of non-phenolic substrates, laccase requires the presence of a mediator in the medium. A mediator is a small molecule that behaves like an 'electron shuttle' between laccase and substrate and these small molecular-mass compounds are converted into stable radicals by means of enzymatic oxidation. They act as redox mediators and oxidize other compounds that, in principle, are not substrates of laccase. From the description of the first laccase mediator, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) to more recent use of the—NOH-type synthetic mediator, including 1-hydroxybenzotriazole (HBT), violuric acid (VLA) and *N*-hydroxyacetanilide (NHA), a large number of studies have been produced on the mechanisms of oxidation of non-phenolic substrates (Baiocco et al. 2003). The use of naturally occurring mediators

would present environmental and economic advantage (Camarero et al. 2005). The enzyme possesses great biotechnological potential because of its wide reaction capabilities as well as broad substrate specificity. Promising applications include biosensors for drug analysis and phenols in tea (Ghindilis et al. 1992; Peter and Wollenberger 1997), polymer synthesis (Huttermann et al. 2001), textile-dye bleaching (Claus et al. 2002), bioremediation (Murugesan 2003; Wesenberg et al. 2003), fungicidals (Spillman 2003) pulp bleaching (Palonen and Viikari 2004), clarification of juices and wines (Ygshinwa 2004).

Distribution of laccases

Laccases are common enzymes in nature. The first laccase was reported in 1883 from *Rhus vernicifera*, the Japanese lacquer tree (Reinhammar 1984), from which the designation laccase was derived. Subsequently laccases have been discovered in numerous other plants, e.g., peach (Lehman et al. 1974), sycamore (Bligny and Douce 1983), tobacco (De Marco and Roubelakis-Angelakis 1997) and poplar (Ranocha et al. 1999). The plant laccases have not been characterized or used extensively despite their wide occurrence, because their detection and purification is often difficult, as the crude plant extracts contain a large number of oxidative enzymes with broad substrate specificities (Ranocha et al. 1999). The majority of laccases characterized so far have been derived from fungi especially from white-rot basidiomycetes that are efficient lignin degraders. Well-known laccase producers also include fungi belonging to the ascomycetes, deuteromycetes, basidiomycetes, and cellulolytic fungi such as *Neurospora crassa* (Froehner and Eriksson 1974), *Agaricus bisporus* (Wood 1980), *Botrytis cinerea* (Marbach et al. 1984), *Pleurotus ostreatus* (Sannia et al. 1986), *Phlebia radiata* (Niku-Paavola et al. 1988), *Trametes versicolor* and *Coriolus polyporus* (Rogalski et al. 1991), *Pycnoporus cinnabarinus* (Eggert et al. 1996), *Chaetomium thermophilum* (Chefetz et al. 1998), and *Coprinus cinereus* (Schneider et al. 1999).

Occurrence of laccases in bacteria

Despite the knowledge about the widespread occurrence of prokaryotic laccases, until now, the complete purification and characterization of only three bacterial laccases have been achieved so far. The first report of prokaryotic laccase is from the rhizospheric bacterium *Azospirillum lipoferum* (Givaudan et al. 1993), where laccase occurs as a multimeric enzyme composed of a catalytic subunit and one or two large chains. The enzyme plays a role in cell pigmentation and utilization of plant phenolic compounds (Faure et al. 1994, 1995) and/or electron transport (Alexandre et al. 1999) (Table 1).

Another laccase has been reported from a melanogenic marine bacterium *Marinomonas mediterranea* producing two different polyphenol oxidases (PPO), an unusual multi-potent PPO able to oxidize substrates characteristic of both tyrosinase and laccase (Solano et al. 1997).

Laccase-like activity has also been found in other bacteria, e.g., CopA protein from *Pseudomonas syringae* (Mellano and Cooksey 1988) and PcoA protein from *Escherichia coli* (Brown et al. 1995). These are structurally homologous to multi-copper oxidases with respect to canonical copper binding sites and have been shown to be important for bacterial copper resistance.

EpoA from *Streptomyces griseus* has been characterized physicochemically and biochemically as a laccase or related enzyme (Endo et al. 2003) and has been expressed as recombinant rEpoA in *E. coli*. It occurs as a homotrimer of 114 kDa and has relatively narrow substrate specificity, as it does not oxidize a number of substrates including guaiacol and syringaldazine, which are known model laccase substrates. The enzyme appears to have a role in morphogenesis in *Streptomyces* spp. (Endo et al. 2002).

A putative multi-copper oxidase encoded by the *yacK* gene of *E. coli* had been cloned and expressed in *E. coli* strain TG-1 containing plasmid pWLFO-5. The expressed enzyme contained the predicted copper centers that harbored both phenol oxidase and ferroxidase activities. The enzyme caused oxidation of a phenolate siderophore that released chelated iron in such a way that the level of free iron was increased in periplasm and as a consequence Fe II and Cu II uptake via *feo* system became appropriately balanced. Such a mechanism conferred a level of protection from elevated copper on the bacterium and left the more soluble form of Fe II available for uptake (Kim et al. 2001).

The best-studied bacterial laccase is the CotA, the endospore coat component of *Bacillus subtilis*. The *cotA* gene codes for a 65-kDa protein belonging to the outer spore coat. CotA participates in the biosynthesis of the brown spore pigment, which is also thought to be a melanin-like product (Driks 2004) and seems to be responsible for most of the protection afforded by the spore coat against UV light and hydrogen peroxide. CotA protein displays similarities with multi-copper oxidases. The protein exhibits a higher thermal stability with a half-life of about 2 h at 80°C and optimum temperature 75°C (Martins et al. 2002). Ligands involved in T1 copper binding are two histidines, a

Table 1 The known bacterial laccases/laccase–like proteins

Species	Possible function	Amino acid length (accession number)	Molecular mass (kDa)	References
Aquifex aeolicus (*sufI*)	Cell division protein	527 (NP_213779)	59.3	Deckert et al. (1998)
Azospirillum lipoferum	Pigmentation, oxidation of phenolic compounds, electron transport	–	Multimeric: 48.9, 97.8, and 179.3	Givaudan et al. (1993)
Bacillus sp. (*mnx* G)	Sporulation, Mn^{2+} oxidation	1217 (AAB06489)	Multimeric	Van Waasbergen et al. (1996)
Bacillus sphaericus	Sporulation, pigmentation	–	–	Claus and Filip (1997)
Bacillus subtilis (*cot*A)	Pigmentation of spores, UV and H_2O_2 resistance	511 (SP P07788)	65	Hullo et al. (2001)
Bacillus halodurans C-125 (*lbh* 2082)	Cu^{2+} resistance	500 (NP_242948)	56	Ruijssenaars and Hartmans (2004)
Escherichia coli (*yac*K)	Cu^{2+} efflux, oxidation of phenolate—siderophores ferrooxidase activity	516 (SP P36649)	56.5	Kim et al. (2001); Roberts et al. (2002)
Leptothrix discophora SS1	Detoxification of Mn^{2+}, destruction of toxic oxygen species	1662 (CAA81037)	110	Lee et al. (1987)
Marinomonas mediterranea (*ppo*A)	Pigmentation	675 (AAF75831)	54.3	Sanchez-Amat and Solano (1997); Sanchez-Amat et al. (2001)
Oceanobacillus iheyensis (*cotA*)	Sporulation	513 (BAC13302)	59.08	Takami et al. (2002)
α-proteobacterium SD 21	Mn^{2+} oxidation	–	150 and 250	Francis and Tebo (2002)
γ-proteobacterium JB	Oxidation of toxic compounds	–	120	Bains et al. (2003)
Pseudomonas fluorescens GB-1	Mn^{2+} oxidation, destruction of toxic oxygen species	–	180 and 250	Okazaki et al. (1997)
Pseudomonas maltophila	Nucleoside oxidase activity	–	–	Isono and Hoshino (1989)
Pseudomonas putida GB1(*cum*A)	Mn^{2+} oxidation	460 (AAD24211.1)	50	Brouwers et al. (1999)
Pseudomonas sp (*cum*A)	Mn^{2+} oxidation	–	50	Francis and Tebo (2001)
Pseudomonas syringae pv .*tomato* (*cop*A)	Cu^{2+} resistance	609 (SP P12374)	67.35	Cha and Cooksey (1991)
Pseudomonas aerophilum (*pae1888*)	Unknown	477 (NP_559612)	52.9	Fitz-Gibbon et al. (2002)
Streptomyces antibioticus	Phenoxazinone synthesis	642 (AAA86668.1)	67.5	Freeman et al. (1993)
Streptomyces griseus (*epo*A) Endo et al. (2002)	Pigmentation, morphogenesis	348 (BAB64332)	114 (Homotrimer)	
Thermus thermophilus HB27	–	462 (BAE16261)	53	Miyazaki (2005)
Xanthomonas campesteris (*copA*)	Cu resistance	635 (AAA72013)	69.5	Lee et al. (1994)

cysteine, and a fourth ligand that varies between the members of the multi-copper oxidase family. In the equivalent sequence position CotA has a methionine residue (Met-502) and, thus, presents a T1 copper site similar to that of ZAO but also to other fungal laccases. The single amino acid substitution of histidine 497 by an alanine (H497A) or replacement of methionine 592 with a leucine (M502L) impair the copper coordination

by the surface-exposed T1 center, thus altering drastically the enzymological properties of the protein.

A laccase-like enzyme activity was also found in spores of *Bacillus sphaericus* (Claus and Filip 1997). A laccase-like spore protein of a marine bacillus strain SG1 has been shown to oxidize Mn (II). Mutants in genes coding for multi-copper oxidases have lost their metal-oxidizing activities. On polyacrylamide gels, laccase activity was present as molecular mass complexes or multimers in enzymes from this bacterium (Van Waasbergen et al. 1996).

Recently, a protein encoded by ORF bh2082 of *Bacillus halodurans* C-125 (Ruijssenaars and Hartmans 2004) has been identified as a potential bacterial laccase by genome mining. The enzyme showed an alkaline pH optimum with syringaldazine as the substrate and was stimulated rather than inhibited by chloride. This finding underlines the potential of bacteria as a source of unusual laccases that may not have some of the disadvantages of the classical laccases. Similarly a non-melanogenic alkalotolerant γ-proteobacterium JB isolated from industrial wastewater drained soil has been shown to produce a pH-stable laccase with no tyrosinase activity. The activity was demonstrated by specific substrate reactions, sensitivity to specific inhibitors and the presence of copper atoms (Bains et al. 2003; Malhotra et al. 2004).

Laccase-homologous sequences identified in thermophiles are very rare. Some of the recent reports include: laccase (NCB Accession No. JC8030) from *Streptomyces lavendulae* REN-7 (Suzuki et al. 2003); a laccase-like protein (NCB Accession No. NC_003364) from the hyperthermophilic archaeon, *Pyrobaculum aerophilum* IM2 (Fitz-Gibbon et al. 2002); a laccase-like protein (NCB Accession No. NC_000918) from the hyperthermophilic bacterium *Aquifex aeolicus* VF5 (Deckert et al. 1998) and laccase (NCB Accession No. AAS81712) from *Thermus thermophilus* HB27 (Miyazaki 2005).

Cellular localization

In plants and fungi, extracellular localization of the enzymes helps them circumvent the problem of the reactive species, such as semiquinones and quinones that are generated by laccases while oxidizing aromatic substrates. These reactive species are powerful inhibitors of the electron transport system in both bacteria and mitochondria. In the case of bacteria, most of the laccases so far studied are located intracellularly as in *A. lipoferum* (Diamantidis et al. 2000), *M. mediterranea* (Solano et al. 1997) and in *B. subtilis* (Martins et al. 2002). The bacterial cells must have some strategy to cope with the intracellular presence of laccase and its toxic by-products. Rearrangement of the electron transport system has been hypothesized to be one of the ways in which the laccase-positive cells adapt to endogenous substituted quinones generated as products of laccase catalyzed reaction. The loss of cytochrome *c* oxidase activity and acquistion of resistance to quinone analogues has been demonstrated in a laccase-positive variant of *A. lipoferum* (Alexandre et al. 1999).

Structural organization

Genetic

Marinomonas mediterranea ppoA is the first prokaryotic laccase sequenced containing laccase and tyrosine activites and first PPO showing such multi-potent catalytic activity. The amino acid sequence shows the existence of a signal peptide and four copper-binding sites characteristics of the blue multi-copper proteins and additional putative copper-binding sites near its N-terminus (Sanchez-Amat et al. 2001). The genetic and molecular basis of copper resistance of *Xanthomonas campestris* pv. *juglandis* have been well studied, and hybridization analysis indicates that the copper resistance genes are located on the chromosomes, sharing nucleotide sequence similarity with other copper resistance genes from *P. syringae* pv. *tomato*, *P. syringae* and *X. campestris* pv. *vesicatoria.* DNA sequence analysis of the fragment revealed four open reading frames (ORF1–ORF4) in the same direction. Four histidine-rich polypeptide regions in the ORF1 of *X. campestris* pv. *juglandis* and copA of *P. syringae* pv. *tomato* strongly resembles the copper-binding motifs of small blue copper proteins and multi-copper oxidases, such as fungal laccases, plant ascorbate oxidase (Asox), and human ceruloplasmin (Lee et al. 1994). *Pseudomonas putida* GB-1 catalyzes the oxidation of Mn^{2+} and nucleotide sequence analysis of the transposon insertion site revealed a gene *cumA* encoding a protein homologous to multi-copper oxidase (Brouwers et al. 1999).

Secondary structure

The structural comparison of bacterial laccases with fungal laccases is done using CotA as a model structure. This is a monomeric protein (Hullo et al. 2001). The primary sequence of CotA was aligned with that of monomeric multi-copper oxidases of known three-dimensional structures: CueO, the laccase CcLa and Asox using Multalin (Fig. 1).

The overall CotA fold comprizes three cupredoxin-like domains, as in case of fungal laccases but all the

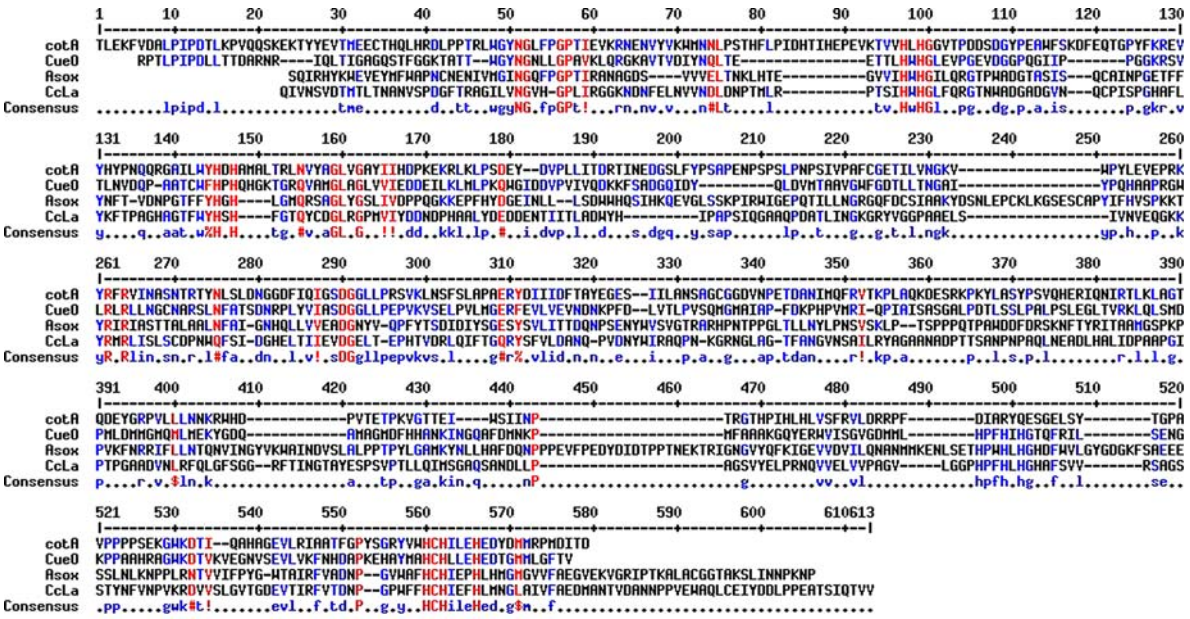

Fig. 1 Sequence alignment by Multalin: CotA (*Bacillus subtilis*), CueO (*Escherichia coli*), Asox (Ascorbate oxidase), CcLa (*Coprinus cinereus*)

three domains are structurally different from fungal and similar to the laccase-like protein of *E. coli* (CueO) (Roberts et al. 2002). The cupredoxin fold is mainly formed by an eight-stranded Greek key β-barrel, comprising two β-sheets composed by four strands, arranged in a sandwich conformation. The first (N-terminal, domain 1 in Fig. 2) cupredoxin-like domain of CotA, residues 2–176, has a somewhat distorted conformation in comparison with the equivalent domain in other multi-copper oxidases. It comprizes of eight strands organized in a β-barrel form, starting with a coiled section (residues 2–25; Fig. 2) that connects domains 1 and 2, and is stabilized by hydrogen bonds, contributing to the packing between these domains. This coiled section is absent in plant and fungal multi-copper oxidases such as the laccase from *C. cinereus* (CcLa) and Asox. However, a similar coiled section present in the *E. coli* CueO protein (Fig. 1).

The overall fold of the second cupredoxin-like domain of CotA (domain 2, in Fig. 2) comprizes a β-barrel composed of 12 strands residues (183–340) very similar to the fold of domain 2 in Asox. Domain 2 of CotA acts as a bridge between domains 1 and 3, but a short α-helical fragment, encompassing residues 177–182, makes the connection between domains 1 and 2, whereas a large loop segment including residues 341–368 links domains 2 and 3. In both the structures of CotA and CueO, this region represents an external connection between domains 2 and 3, whereas in plant and fungal multi-copper oxidases the corresponding link is made through an internal connection. Therefore, this feature may be a characteristic of the prokaryotic variants of these enzymes. Together with the coiled section that links domains 1 and 2 of CotA (which also has an equivalent in CueO; residue 169–174 in Fig. 1), this external loop motif contributes decisively to the closer resemblance between the overall folds of the prokaryotic proteins CotA and CueO, relative to other structurally characterized multi-copper oxidases.

Finally, domain 3 of CotA (Fig. 2; residues 369–501) not only contains the mononuclear copper center, but also contributes to the formation of the binding site of the trinuclear copper center, which is located in the interface between domains 1 and 3 (Fig. 2). Moreover, domain 3 includes the putative substrate-binding site, located at the surface of the protein, close to the type I mononuclear copper center. A protruding section formed by a loop and short α-helix (Fig. 2), comprising amino acids from 434 to 454, forms a lid-like structure over the substrate-binding site. No similar element has been found in the previously analyzed multi-copper oxidases with the known three-dimensional structure. Therefore, this structural element represents a distinctive feature of bacterial laccases. The laccase from *B. subtilis* (Martins et al. 2002) and *S. lavendulae* (Suzuki et al. 2003) have 47% similarity to each other but are different from other laccases.

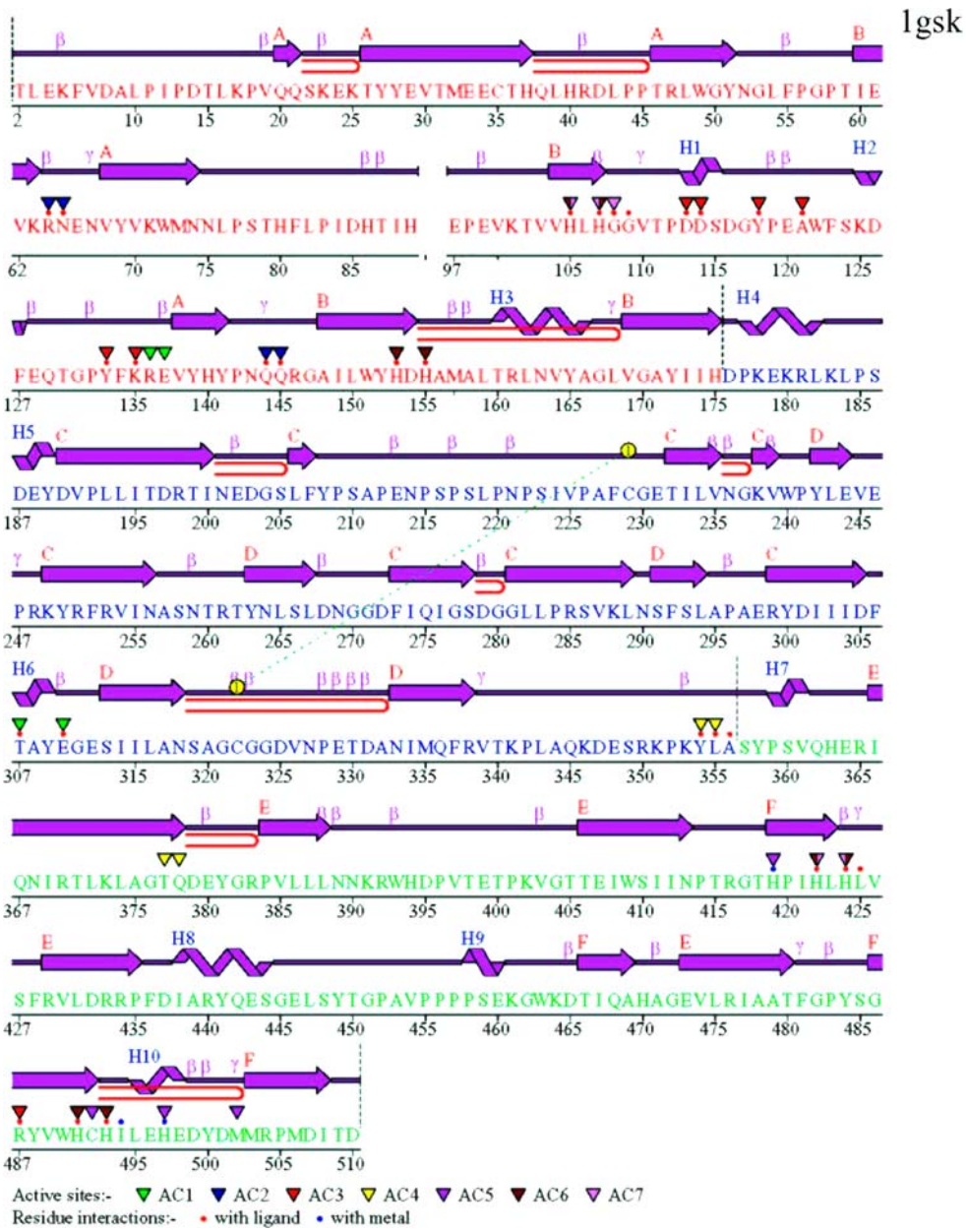

Fig. 2 Secondary structure of *Bacillus subtilis* CotA (PDB code 1GSK)

Protein characterization

Putative laccase–like multi-copper oxidases have been detected in the genomes of many bacterial species, suggesting that laccases are widespread in bacteria (Alexandre and Zhulin 2000). However, of three laccases that have been structurally characterized, those from *C. cinereus* (Ducros et al. 1998), from *T. versicolor* (Bertrand et al. 2002) and from *Melanocarpus albomyces* (Hakulinen et al. 2002) none is of bacterial origin. The first three-dimensional structure on bacterial laccase was reported for *B. subtilis* CotA laccase as determined by X-ray crystallography. The 65-kDa CotA protein is a highly thermostable laccase which is associated with high-proline content. CotA contains 46 proline residues, a number that does not differ greatly from that observed for other laccases. Atomic packing has been recognized as an important measurement for characterizing protein structure. Several methods have been defined for determining atomic packing, and OSP values for CotA protein showed three intervals in which this value corresponded to a more packed structure. These highly packed residues are localized in the interface regions between domains 1 and 2 and close to the mononuclear copper center (Fig. 3). Together, they constitute a pocket around the copper

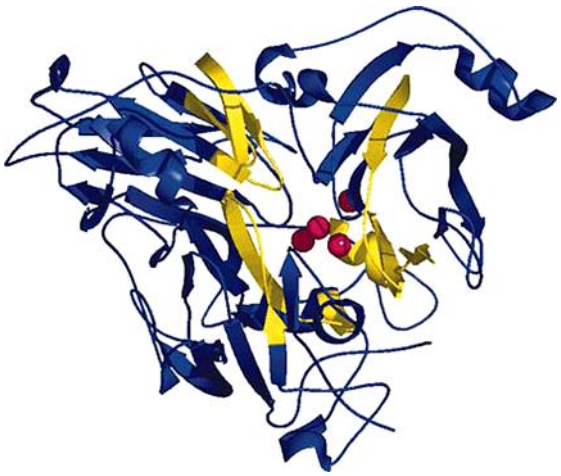

Fig. 3 Ribbon representation of the CotA structure, with the localization of the residues with higher OSP values (represented in yellow). Copper atoms are represented as magenta balls

atoms and contribute to the overall structure packing of CotA protein (Enguita et al. 2003).

Catalytic properties

The list of substrates oxidized by laccases has increased significantly in recent years: methoxy- or amino-monophenols and several non-phenolic compounds such as aromatic diamines, ABTS, 1-naphthol, hydroxyindoles and syringaldazine are laccase substrates (Mayer 1987; Cai et al. 1993). Moreover, in the presence of ABTS, laccases are able to oxidize several compounds, which are not laccase substrates on their own (Bourbonnais and Paice 1992).

Fungal and plant laccases have been extensively described by several substrates and inhibitors acting on them, but little data on bacterial laccase activity has been reported. Faure et al. (1995) compared commercial fungal laccase and catechol oxidase, purified from *Pyricularia oryzae* and *A. bisporus*, respectively, with bacterial laccase from *A. lipoferum* by using several substrates and phenol oxidase inhibitors. Five classes of chemical compounds were investigated as substrates for laccase: (1) L-tyrosine and several substituted monophenols such as *p*-coumaric and *o*-hydroxyphenylacetic or salicylic acids; (2) *o*-diphenols (catechol, pyrogallol, guaiacol, and protocatechic, gallic, and caffeic acids), L-3,4-dihydroxyphenylalanine and *o*-aminophenol, which could be oxidized by both laccase and catechol oxidase; (3) *p*-diphenol and *p*-substituted aromatic compounds as typical *p*-phenol oxidase (*p*-PO) substrates such as hydroquinone, *p*-cresol, *p*-aminophenol and *p*-phenylenediamine; (4) *m*-diphenols such as resorcinol, orcinol, 4-hexylresorcinol, and 5-pentadecylresorcinol and (5) other laccase substrates such as syringaldazine, 1-naphthol, ABTS, and 4- and 5-hydroxyindoles. The range of substrates used by *A. lipoferum* laccase was similar to that used by *P. oryzae* laccase.

Laccase signature sequences

Identification of putative bacterial laccases has been done *in silico* using fungal laccase sequences as queries. The bacterial protein sequence database and unfinished microbial genomes (http://www.tigr.org) have been searched. It is possible that laccases, due to their comparatively broader substrate specificities (Xu 1996), share a sequence signature that can distinguish them as a specific subgroup of (Bourbonnais and Paice 1990; Xu et al. 1996) the muti-copper oxidase family. Based on the pattern of conservation noted for some of the amino acid ligands at the T1 copper center, a 21 residue signature sequence (Mann et al. 1988; Messerchmidt and Huber 1990; Ouzounis and Sander 1991; Askwith et al. 1994) was defined for the multi-copper oxidases as G-X-[FYW]-X[LIVMFYW]-X-[CST]-X_8-G-[LM]-X_3-[LIVMVYM]. An X in this signature represents an undefined residue while the multiple letters within brackets represent a partially conserved residue. The amino acid ligands of the trinuclear cluster are the eight histidine, which occur, in a highly conserved pattern of 4HXH motifs in the enzyme as shown in (Fig. 1 in red color). The HXH motifs are separated from one another by segments of between 25 and 175 residues and are likely to be brought close in composite catalytic apparatus by protein folding. An additional 12 residue long type II signature sequence has been defined as HCH-X_3-H-X_3-[AG]-[LM] as seen in Fig. 1 (from residues 560–571). Analysis of over 100 plant and fungal laccases resulted in identification of a sequence signature that uniquely characterizes the laccases as a distinctive subgroup of enzymes of the multi-copper oxidase family (Table 2). The signature, composed of four ungapped sequence segments L1–L4 ranging from 8 to 24 residues in length and scattered across almost the entire length of the protein, contains all the amino acid ligands of the copper centers and other completely or partially conserved residues, of which L2 and L4 conform to the earlier reported copper signature sequences of multi-copper oxidases while L1 and L3 are distinctive to laccases (Kumar et al. 2003). However, the signature sequences have not as yet been reported in bacterial laccases.

Table 2 Signature sequences

Multi-copper oxidase signature sequences		
Type I	G-X-(FYW)-X-(LIVMFYW)-X-(CST)-X_8-G-(LM)-X_3-(LIVMFW)	Mann et al. (1988); Messerschmidt and Huber (1990); Ouzounis and Sander (1991)
Type II	H-C-H-X_3-H-X_3-(AG)-(LM)	Askwith et al. (1994)
Laccase signature sequences from fungal and plant laccases		
L1	H-W-H-G-X_9-QCPI	
L2	G-T-X-W-Y-H-S-H-X_3-Q-Y-C-X-D-G-L-X-G-X- (FLIM)	Kumar et al. (2003)
L3	H-P-X-H-L-H-G-H	
L4	G-(PA)-W-X-(LFV)-HCHI-DAE-X-H-X_3-G-(LMF)-X_3-(LFM)	

Applications

Laccases are well-known biocatalysts in transforming phenolic or aromatic amines and are considered to be some of the most promising enzymes for future industrial applications (Xu 2005). In particular, laccases from fungi have found wide applications ranging from the pharmaceutical sector to the pulp and paper industry to reduce the kappa number and enhance the bleaching of kraft pulp when they are used in the presence of chemical mediators, such as ABTS (Bourbonnais and Paice 1996). Bio-bleaching eliminates the use of chlorine in the bleaching process of pulp mills and thus stops the generation of chlorinated toxic pollutants. Identification of bacterial laccases for which genetic tools and biotechnological processes are well established may be of significant importance for applications, but there are very few reports on applications of bacterial laccases. Bacterial laccases from *Streptomyces cyaneus* CECT 3335 (Arias et al. 2003) and *Pseudomonas stutzeri* (Kumar et al. 2005) have been evaluated for biobleaching of eucalyptus kraft pulps by using ABTS and HOBT as mediators. In contrast to fungal laccases, bacterial laccases are overcoming the disadvantages of instability and in-process applications. They are highly active and much more stable at high temperatures and high-pH values. For many continuous process applications it is necessary that the catalysts used are kept in the process via immobilization or membrane reactors. The immobilized spore laccases can also be used for the continuous biotransformation of any laccase substrate (Held et al. 2005). Due to the high temperature and pH stability these immobilized spores are compatible with almost all industrial processes.

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