



Contributions to the molecular characterisation of a versatile peroxidase from a novel *Bjerkandera* strain



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INTRODUCTION

A novel class of lignolytic peroxidases, named versatile peroxidases (VP), with high affinity for manganese and dyes, has been described. A novel fungal strain was identified in our laboratory as belonging to the *Bjerkandera* genus, and was tentatively named *Bjerkandera* sp. strain B33/3. One of the main enzymes that are associated with its dye decolouring activity exhibited also properties characteristic of the novel class of peroxidases with ability to oxidise manganese, as well as veratryl alcohol and 2,6-dimethoxyphenol in a manganese-independent reaction. This new enzyme, which belongs to the novel versatile peroxidase class, was named RBP.

The objectives of the present study were to clone the gene encoding the RBP versatile peroxidase, and duly characterise it.

MATERIALS AND METHODS

DNA and RNA were extracted from 6 day-old mycelium grown on CDBYE medium, as previously described [1]. The RNA isolation was conducted with the SV Total RNA Isolation System (Promega). cDNA was prepared from total RNA with ImProm-II TM Reverse Transcription System (Promega). With the purpose of cloning the gene that codifies for the RBP versatile peroxidase, the oligonucleotides F-Peroxi (N-terminal) and Cterm5, complementary to the 3' end of the cDNA sequence were used in a PCR experiment on the total genomic DNA. Sequencing reactions were carried out with an ALF Express II DNA sequencer (Amersham Pharmacia Biosciences). The PSI-BLAST 2.2.8 (NCBI http server), and BioEdit Sequence Alignment Editor programs were used for analysis, alignment and comparison of sequences of both amino acids, DNA and cDNA sequences. A dendrogram of affinities between sequences of mature peroxidases was obtained using the Neighbour Joining method, from the Kimura distance matrix obtained from optimal pairwise multiple alignment of sequences using BioEdit Sequence Alignment Editor programs and PHYLIP (phylogenetic inference package) programs.

RESULTS AND DISCUSSION

The sequence of the 1771 bp DNA fragment isolated during this work received the accession number AY217015 in the Genbank database.

On the basis of the existing homology of said sequence with the cDNA previously obtained and coupled with the GT-AG rule of the intron splice sequences, it was possible to predict its intron/exon structure. Comparison of these sequences revealed a peroxidase gene consisting of 1698 bp of protein-encoding DNA, that is interrupted by 597 bp of intervening sequences. The intron splice junction sequence formation sites strictly adhere to the GT-AG rule, and all of the putative internal Lariat formation sites conform to the NNHTNAY rule.

Table 1 Amino acid sequence of the RBP versatile peroxidase from *Bjerkandera* sp. strain B33/3 deduced from *rbp* gene. The 367 amino acid deduced sequence includes a 27 amino acid signal peptide.

AMFKQLTAM	LSLALVLPFS	QAMTRAVAC	PDGVNTATNA	ACCALFAVRD	-27
DIQQNLFDDGG	EGG EVH SL	RLTFHVAIGI	SPSLAATGKF	GGG ADG IM	24
IFDDIIPNFH	ANNGVDEIIN	AQKPFVAKHN	MTAGDFIQFA	GAVGVSNCPG	74
APQLSFFLGR	PAATQPAPDG	LVPEPFDSVT	DILNRFADAG	GFTIQEVVWL	124
LASHIAAA	HVDPTIPGSP	FASTPEFAT	QFFVETLLKG	VLFPGTSGNQ	174
GEVESPLAGE	IRLQSDADF	RDSRTACEWQ	SFVNNQPRMQ	VLFKAAAMQKL	224
SILGHDLTQM	IDCSDVIPVP	PSTAVRGSHL	PAGNTLDDIE	QACASTPFPS	274
LTADPGPATS	VAPVPPS				324

The 367 amino acid deduced-sequence that is depicted in Table 1 includes a 27 amino acid signal peptide. This table highlights heme pocket residues (R44, F47, H48, E89, N95, H177, F194 and D249), that include both distal and proximal histidine residues, H48 and H177. There are also calcium potential binding residues (D49, D47, D49, D71, D178, D195, D198), and 8 cysteine residues, characteristic of other fungal peroxidases. The sequence also includes residues involved in substrate oxidation, three acid residues (E87, E47, and D195) putatively involved in manganese binding, and H83, F149, W173 and A240 putatively involved in oxidation of aromatic substrates. Peroxidase RBP does not contain tyrosine residues. The sequence includes one potential N-glycosylation site (N103) according to NXS/T consensus, which could account for the 5.6% N-linked carbohydrate.

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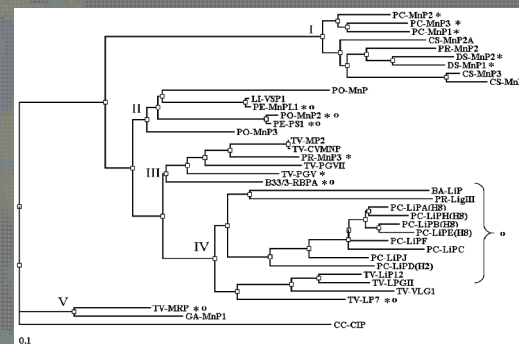


Figure 1. Sequence comparison of mature fungal peroxidases. The dendrogram was built from Kimura distances between mature proteins using PHYLIP software package after multiple alignment of 38 fungal peroxidase sequences, and Neighbour-Joining Method from PHYLIP software package. The presence of manganese binding site (*) and exposed tryptophan involved in veratryl alcohol oxidation (O) are duly indicated.

As shown in Figure 1, most classical MnPs are clustered together (Group I), as well as most classical LiPs (Group II). Versatile peroxidases appear in two separate groups (Group II and III), where *Pleurotus* VP appears together, and where RBP is associated with manganese peroxidases and other peroxidases from *Trametes* sp. not clustered in group I.

CONCLUSION

The results reported herein indicate that the enzyme produced by the *Bjerkandera* sp. strain B33/3, of which some biochemical and molecular characteristics were highlighted, can be included, together with those produced by *B. adusta* and *Pleurotus* sp., in a new class of versatile peroxidases, which share catalytic properties of both LiP and MnP.

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