Molecules **2016**, *21*, 203; doi:10.3390/molecules21020203 S1 of S3

Supplementary Materials: Biochemical Characteristics of Three Laccase Isoforms from the Basidiomycete *Pleurotus nebrodensis*

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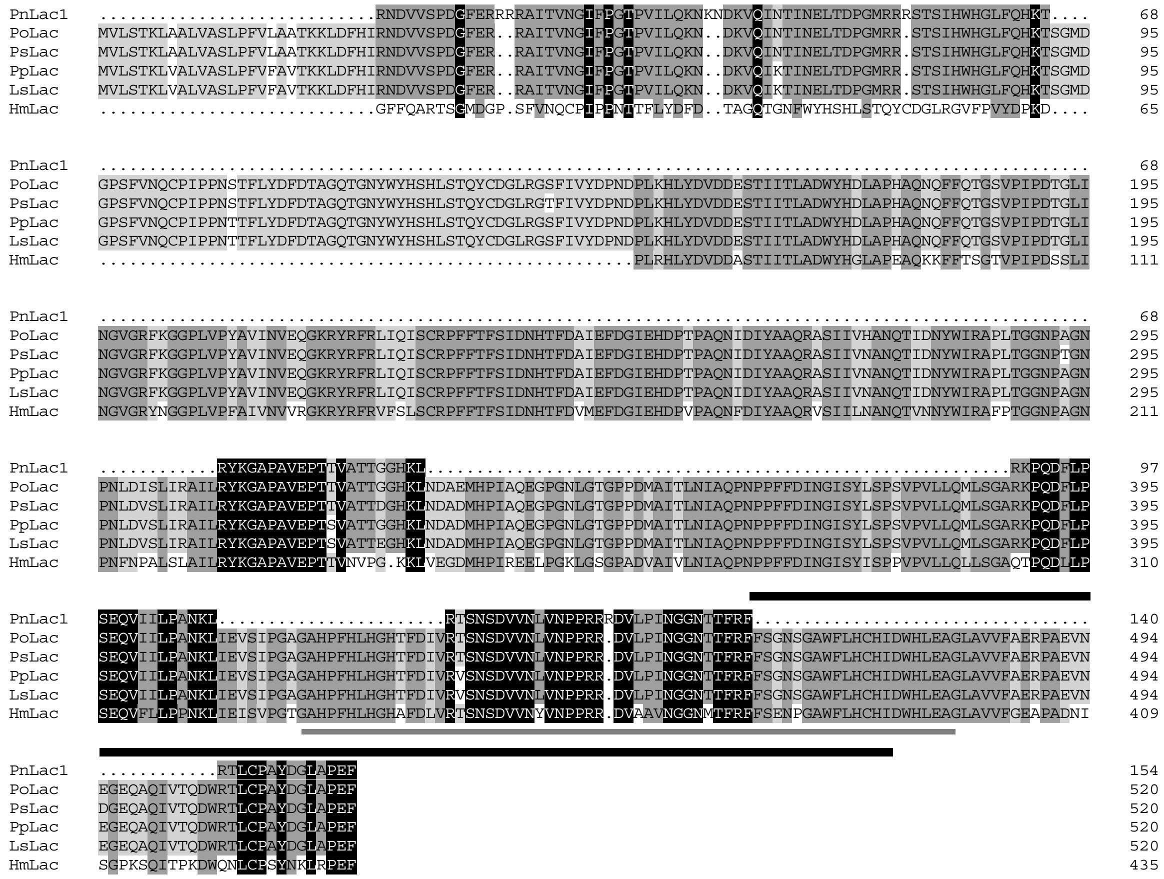

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PsLac    MVLSTKLAALVASLPFVLAATKKLDFHIRNDVVSPDGFER..RAITVNGIFPGTPVILQKN..DKVQINTINELTDPGMRR.STSIHWHGLFQHKTSGMD    95
PpLac    MVLSTKLVALVASLPFVFAVTKKLDFHIRNDVVSPDGFER..RAITVNGIFPGTPVILQKN..DKVQIKTINELTDPGMRR.STSIHWHGLFQHKTSGMD    95
LsLac    MVLSTKLVALVASLPFVFAVTKKLDFHIRNDVVSPDGFER..RAITVNGIFPGTPVILQKN..DKVQIKTINELTDPGMRR.STSIHWHGLFQHKTSGMD    95
HmLac    ............................GFFQARTSGMDGP.SFVNQCPIPPNTTFLYDFD..TAGQTGNFWYHSHLSTQYCDGLRGVFPVYDPKD....    65

PnLac1   ....................................................................................................    68
PoLac    GPSFVNQCPIPPNSTFLYDFDTAGQTGNYWYHSHLSTQYCDGLRGSFIVYDPNDPLKHLYDVDDESTIITLADWYHDLAPHAQNQFFQTGSVPIPDTGLI   195
PsLac    GPSFVNQCPIPPNSTFLYDFDTAGQTGNYWYHSHLSTQYCDGLRGTFIVYDPNDPLKHLYDVDDESTIITLADWYHDLAPHAQNQFFQTGSVPIPDTGLI   195
PpLac    GPSFVNQCPIPPNTTFLYDFDTAGQTGNYWYHSHLSTQYCDGLRGSFIVYDPNDPLKHLYDVDDESTIITLADWYHDLAPHAQNQFFQTGSVPIPDTGLI   195
LsLac    GPSFVNQCPIPPNTTFLYDFDTAGQTGNYWYHSHLSTQYCDGLRGSFIVYDPNDPLKHLYDVDDESTIITLADWYHDLAPHAQNQFFQTGSVPIPDTGLI   195
HmLac    ......................................................PLRHLYDVDDASTIITLADWYHGLAPEAQKKFFTSGTVPIPDSSLI   111

PnLac1   ....................................................................................................    68
PoLac    NGVGRFKGGPLVPYAVINVEQGKRYRFRLIQISCRPFFTFSIDNHTFDAIEFDGIEHDPTPAQNIDIYAAQRASIIVHANQTIDNYWIRAPLTGGNPAGN   295
PsLac    NGVGRFKGGPLVPYAVINVEQGKRYRFRLIQISCRPFFTFSIDNHTFDAIEFDGIEHDPTPAQNIDIYAAQRASIIVNANQTIDNYWIRAPLTGGNPTGN   295
PpLac    NGVGRFKGGPLVPYAVINVEQGKRYRFRLIQISCRPFFTFSIDNHTFDAIEFDGIEHDPTPAQNIDIYAAQRASIIVNANQTIDNYWIRAPLTGGNPAGN   295
LsLac    NGVGRFKGGPLVPYAVINVEQGKRYRFRLIQISCRPFFTFSIDNHTFDAIEFDGIEHDPTPAQNIDIYAAQRASIIVNANQTIDNYWIRAPLTGGNPAGN   295
HmLac    NGVGRYNGGPLVPFAIVNVVRGKRYRFRVFSLSCRPFFTFSIDNHTFDVMEFDGIEHDPVPAQNFDIYAAQRVSIILNANQTVNNYWIRAFPTGGNPAGN   211

PnLac1   ............RYKGAPAVEPTTVATTGGHKL...........................................................RKPQDFLP    97
PoLac    PNLDISLIRAILRYKGAPAVEPTTVATTGGHKLNDAEMHPIAQEGPGNLGTGPPDMAITLNIAQPNPPFFDINGISYLSPSVPVLLQMLSGARKPQDFLP   395
PsLac    PNLDVSLIRAILRYKGAPAVEPTTVATTDGHKLNDADMHPIAQEGPGNLGTGPPDMAITLNIAQPNPPFFDINGISYLSPSVPVLLQMLSGARKPQDFLP   395
PpLac    PNLDVSLIRAILRYKGAPAVEPTSVATTGGHKLNDADMHPIAQEGPGNLGTGPPDMAITLNIAQPNPPFFDINGISYLSPSVPVLLQMLSGARKPQDFLP   395
LsLac    PNLDVSLIRAILRYKGAPAVEPTSVATTEGHKLNDADMHPIAQEGPGNLGTGPPDMAITLNIAQPNPPFFDINGISYLSPSVPVLLQMLSGARKPQDFLP   395
HmLac    PNFNPALSLAILRYKGAPAVEPTTVNVPG.KKLVEGDMHPIREELPGKLGSGPADVAIVLNIAQPNPPFFDINGISYISPPVPVLLQLLSGAQTPQDLLP   310

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PsLac    SEQVIILPANKLIEVSIPGAGAHPFHLHGHTFDIVRTSNSDVVNLVNPPRR.DVLPINGGNTTFRFFSGNSGAWFLHCHIDWHLEAGLAVVFAERPAEVN   494
PpLac    SEQVIILPANKLIEVSIPGAGAHPFHLHGHTFDIVRVSNSDVVNLVNPPRR.DVLPINGGNTTFRFFSGNSGAWFLHCHIDWHLEAGLAVVFAERPAEVN   494
LsLac    SEQVIILPANKLIEVSIPGAGAHPFHLHGHTFDIVRVSNSDVVNLVNPPRR.DVLPINGGNTTFRFFSGNSGAWFLHCHIDWHLEAGLAVVFAERPAEVN   494
HmLac    SEQVFLLPPNKLIEISVPGTGAHPFHLHGHAFDLVRTSNSDVVNYVNPPRR.DVAAVNGGNMTFRFFSENPGAWFLHCHIDWHLEAGLAVVFGEAPADNI   409

PnLac1   ............RTLCPAYDGLAPEF   154
PoLac    EGEQAQIVTQDWRTLCPAYDGLAPEF   520
PsLac    DGEQAQIVTQDWRTLCPAYDGLAPEF   520
PpLac    EGEQAQIVTQDWRTLCPAYDGLAPEF   520
LsLac    EGEQAQIVTQDWRTLCPAYDGLAPEF   520
HmLac    SGPKSQITPKDWQNLCPSYNKLRPEF   435
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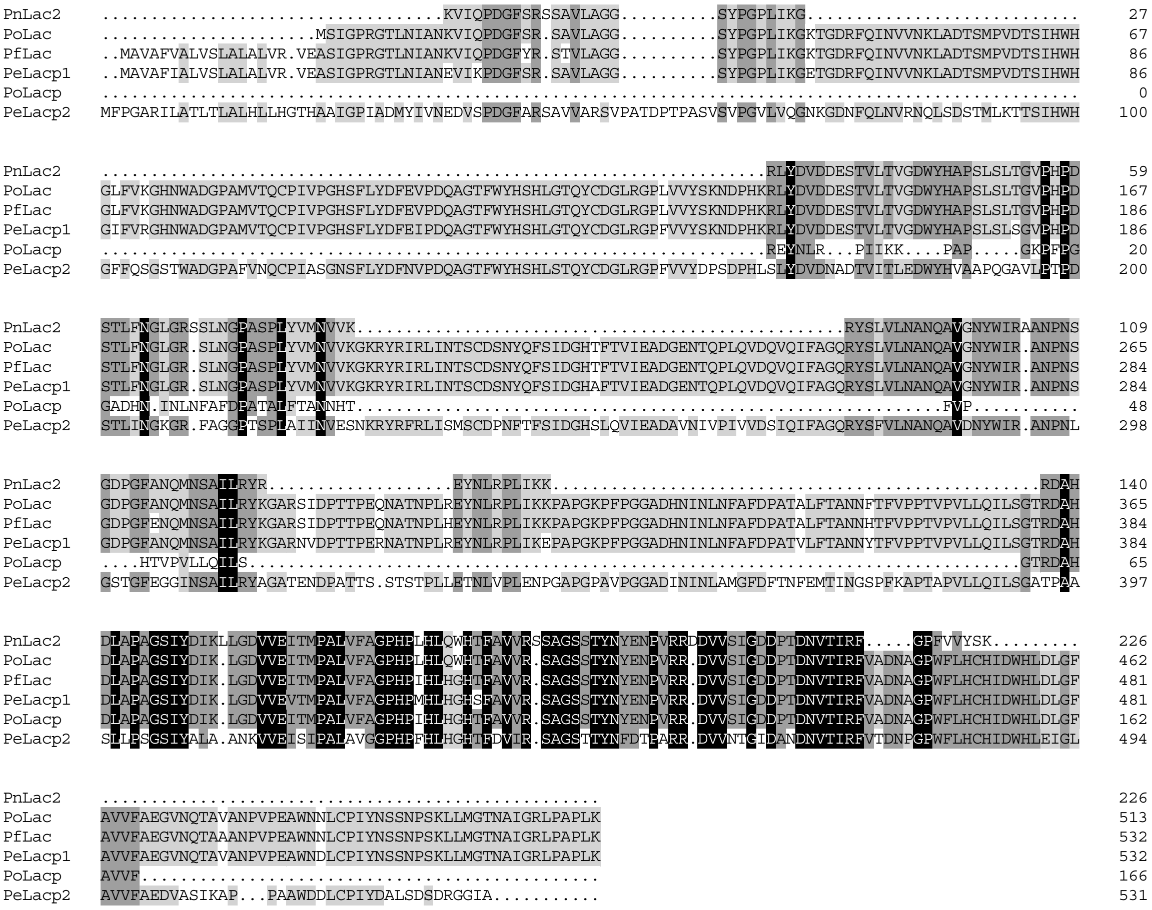
(**a**)

Figure S1. *Cont.*



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PoLac     ......................MSIGPRGTLNIANKVIQPDGFSR.SAVLAGG..........SYPGPLIKGKTGDRFQINVVNKLADTSMPVDTSIHWH    67
PfLac     ..MAVAFVALVSLALALVR.VEASIGPRGTLNIANKVIQPDGFYR.STVLAGG..........SYPGPLIKGKTGDRFQINVVNKLADTSMPVDTSIHWH    86
PeLacp1   ..MAVAFIALVSLALALVR.VEASIGPRGTLNIANEVIKPDGFSR.SAVLAGG..........SYPGPLIKGETGDRFQINVVNKLADTSMPVDTSIHWH    86
PoLacp    ....................................................................................................     0
PeLacp2   MFPGARILATLTLALHLLHGTHAAIGPIADMYIVNEDVSPDGFARSAVVARSVPATDPTPASVSVPGVLVQGNKGDNFQLNVRNQLSDSTMLKTTSIHWH   100

PnLac2    ....................................................................RLYDVDDESTVLTVGDWYHAPSLSLTGVPHPD    59
PoLac     GLFVKGHNWADGPAMVTQCPIVPGHSFLYDFEVPDQAGTFWYHSHLGTQYCDGLRGPLVVYSKNDPHKRLYDVDDESTVLTVGDWYHAPSLSLTGVPHPD   167
PfLac     GLFVKGHNWADGPAMVTQCPIVPGHSFLYDFEVPDQAGTFWYHSHLGTQYCDGLRGPLVVYSKNDPHKRLYDVDDESTVLTVGDWYHAPSLSLTGVPHPD   186
PeLacp1   GIFVRGHNWADGPAMVTQCPIVPGHSFLYDFEIPDQAGTFWYHSHLGTQYCDGLRGPFVVYSKNDPHKRLYDVDDESTVLTVGDWYHAPSLSLSGVPHPD   186
PoLacp    ....................................................................REYNLR...PIIKK....PAP.....GKPFPG    20
PeLacp2   GFFQSGSTWADGPAFVNQCPIASGNSFLYDFNVPDQAGTFWYHSHLSTQYCDGLRGPFVVYDPSDPHLSLYDVDNADTVITLEDWYHVAAPQGAVLPTPD   200

PnLac2    STLFNGLGRSSLNGPASPLYVMNVVK..................................................RYSLVLNANQAVGNYWIRAANPNS   109
PoLac     STLFNGLGR.SLNGPASPLYVMNVVKGKRYRIRLINTSCDSNYQFSIDGHTFTVIEADGENTQPLQVDQVQIFAGQRYSLVLNANQAVGNYWIR.ANPNS   265
PfLac     STLFNGLGR.SLNGPASPLYVMNVVKGKRYRIRLINTSCDSNYQFSIDGHTFTVIEADGENTQPLQVDQVQIFAGQRYSLVLNANQAVGNYWIR.ANPNS   284
PeLacp1   STLFNGLGR.SLNGPASPLYVMNVVKGKRYRIRLINTSCDSNYQFSIDGHAFTVIEADGENTQPLQVDQVQIFAGQRYSLVLNANQAVGNYWIR.ANPNS   284
PoLacp    GADHN.INLNFAFDPATALFTANNHT...........................................................FVP............    48
PeLacp2   STLINGKGR.FAGGPTSPLAIINVESNKRYRFRLISMSCDPNFTFSIDGHSLQVIEADAVNIVPIVVDSIQIFAGQRYSFVLNANQAVDNYWIR.ANPNL   298

PnLac2    GDPGFANQMNSAILRYR...................EYNLRPLIKK..................................................RDAH   140
PoLac     GDPGFANQMNSAILRYKGARSIDPTTPEQNATNPLREYNLRPLIKKPAPGKPFPGGADHNINLNFAFDPATALFTANNFTFVPPTVPVLLQILSGTRDAH   365
PfLac     GDPGFENQMNSAILRYKGARSIDPTTPEQNATNPLHEYNLRPLIKKPAPGKPFPGGADHNINLNFAFDPATALFTANNHTFVPPTVPVLLQILSGTRDAH   384
PeLacp1   GDPGFANQMNSAILRYKGARNVDPTTPERNATNPLREYNLRPLIKEPAPGKPFPGGADHNINLNFAFDPATVLFTANNYTFVPPTVPVLLQILSGTRDAH   384
PoLacp    ....HTVPVLLQILS...............................................................................GTRDAH    65
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PnLac2    DLAPAGSIYDIKLLGDVVEITMPALVFAGPHPLHLQWHTFAVVRSSAGSSTYNYENPVRRDDVVSIGDDPTDNVTIRF.....GPFVVYSK.........   226
PoLac     DLAPAGSIYDIK.LGDVVEITMPALVFAGPHPLHLQWHTFAVVR.SAGSSTYNYENPVRR.DVVSIGDDPTDNVTIRFVADNAGPWFLHCHIDWHLDLGF   462
PfLac     DLAPAGSIYDIK.LGDVVEITMPALVFAGPHPIHLHGHTFAVVR.SAGSSTYNYENPVRR.DVVSIGDDPTDNVTIRFVADNAGPWFLHCHIDWHLDLGF   481
PeLacp1   DLAPAGSIYDIK.LGDVVEVTMPALVFAGPHPMHLHGHSFAVVR.SAGSSTYNYENPVRR.DVVSIGDDPTDNVTIRFVADNAGPWFLHCHIDWHLDLGF   481
PoLacp    DLAPAGSIYDIK.LGDVVEITMPALVFAGPHPIHLHGHTFAVVR.SAGSSTYNYENPVRR.DVVSIGDDPTDNVTIRFVADNAGPWFLHCHIDWHLDLGF   162
PeLacp2   SLLPSGSIYALA.ANKVVEISIPALAVGGPHPFHLHGHTFDVIR.SAGSTTYNFDTPARR.DVVNTGIDANDNVTIRFVTDNPGPWFLHCHIDWHLEIGL   494

PnLac2    ...................................................                                                   226
PoLac     AVVFAEGVNQTAVANPVPEAWNNLCPIYNSSNPSKLLMGTNAIGRLPAPLK                                                   513
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PeLacp1   AVVFAEGVNQTAVANPVPEAWNDLCPIYNSSNPSKLLMGTNAIGRLPAPLK                                                   532
PoLacp    AVVF...............................................                                                   166
PeLacp2   AVVFAEDVASIKAP...PAAWDDLCPIYDALSDSDRGGIA...........                                                   531
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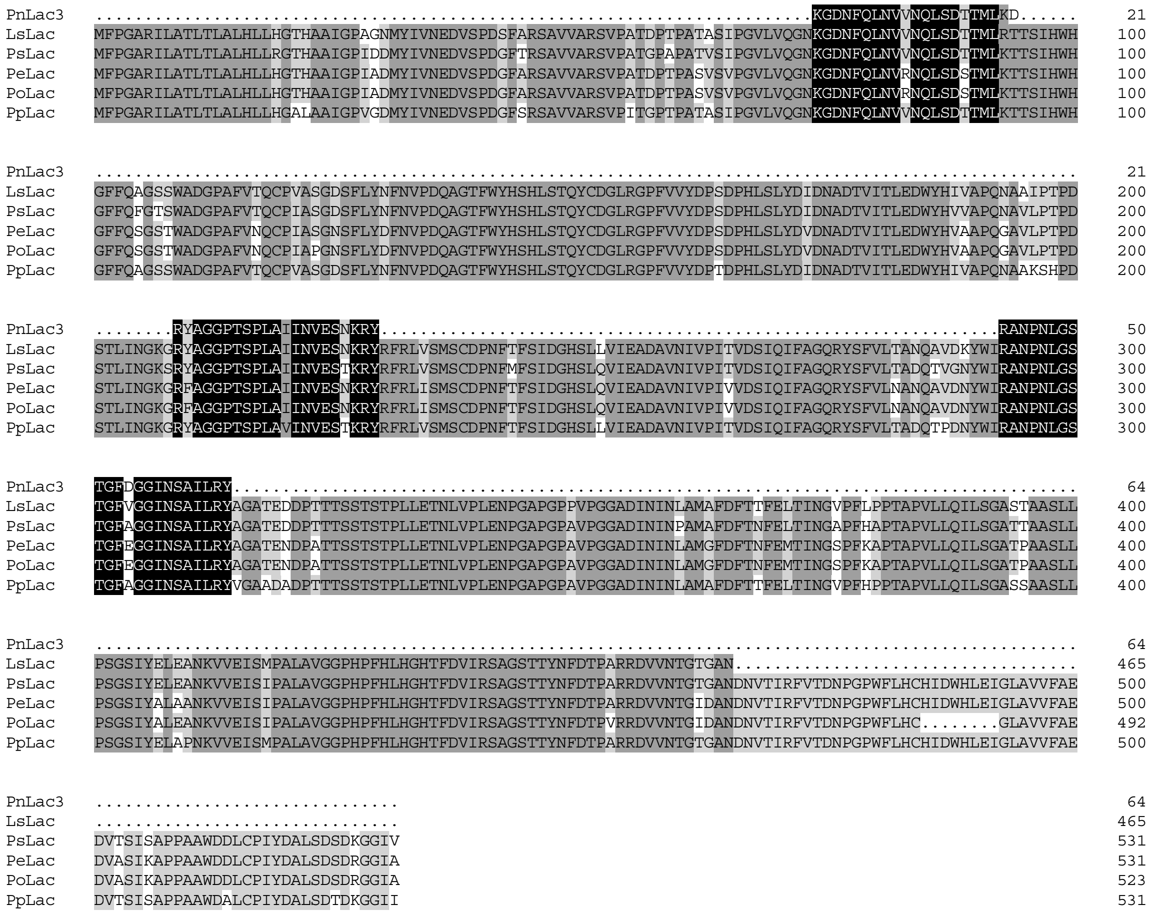
(**b**)

Figure S1. *Cont.*



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PsLac    MFPGARILATLTLALHLLRGTHAAIGPIDDMYIVNEDVSPDGFTRSAVVARSVPATGPAPATVSIPGVLVQGNKGDNFQLNVVNQLSDTTMLKTTSIHWH   100
PeLac    MFPGARILATLTLALHLLHGTHAAIGPIADMYIVNEDVSPDGFARSAVVARSVPATDPTPASVSVPGVLVQGNKGDNFQLNVRNQLSDSTMLKTTSIHWH   100
PoLac    MFPGARILATLTLALHLLHGTHAAIGPIADMYIVNEDVSPDGFARSAVVARSVPATDPTPASVSVPGVLVQGNKGDNFQLNVRNQLSDSTMLKTTSIHWH   100
PpLac    MFPGARILATLTLALHLLHGALAAIGPVGDMYIVNEDVSPDGFSRSAVVARSVPITGPTPATASIPGVLVQGNKGDNFQLNVVNQLSDTTMLKTTSIHWH   100

PnLac3   ....................................................................................................    21
LsLac    GFFQAGSSWADGPAFVTQCPVASGDSFLYNFNVPDQAGTFWYHSHLSTQYCDGLRGPFVVYDPSDPHLSLYDIDNADTVITLEDWYHIVAPQNAAIPTPD   200
PsLac    GFFQFGTSWADGPAFVTQCPIASGDSFLYNFNVPDQAGTFWYHSHLSTQYCDGLRGPFVVYDPSDPHLSLYDIDNADTVITLEDWYHVVAPQNAVLPTPD   200
PeLac    GFFQSGSTWADGPAFVNQCPIASGNSFLYDFNVPDQAGTFWYHSHLSTQYCDGLRGPFVVYDPSDPHLSLYDVDNADTVITLEDWYHVAAPQGAVLPTPD   200
PoLac    GFFQSGSTWADGPAFVNQCPIAPGNSFLYDFNVPDQAGTFWYHSHLSTQYCDGLRGPFVVYDPSDPHLSLYDVDNADTVITLEDWYHVAAPQGAVLPTPD   200
PpLac    GFFQAGSSWADGPAFVTQCPVASGDSFLYNFNVPDQAGTFWYHSHLSTQYCDGLRGPFVVYDPTDPHLSLYDIDNADTVITLEDWYHIVAPQNAAKSHPD   200

PnLac3   ........RYAGGPTSPLAIINVESNKRY...............................................................RANPNLGS    50
LsLac    STLINGKGRYAGGPTSPLAIINVESNKRYRFRLVSMSCDPNFTFSIDGHSLLVIEADAVNIVPITVDSIQIFAGQRYSFVLTANQAVDKYWIRANPNLGS   300
PsLac    STLINGKSRYAGGPTSPLAIINVESTKRYRFRLVSMSCDPNFMFSIDGHSLQVIEADAVNIVPITVDSIQIFAGQRYSFVLTADQTVGNYWIRANPNLGS   300
PeLac    STLINGKGRFAGGPTSPLAIINVESNKRYRFRLISMSCDPNFTFSIDGHSLQVIEADAVNIVPIVVDSIQIFAGQRYSFVLNANQAVDNYWIRANPNLGS   300
PoLac    STLINGKGRFAGGPTSPLAIINVESNKRYRFRLISMSCDPNFTFSIDGHSLQVIEADAVNIVPIVVDSIQIFAGQRYSFVLNANQAVDNYWIRANPNLGS   300
PpLac    STLINGKGRYAGGPTSPLAVINVESTKRYRFRLVSMSCDPNFTFSIDGHSLLVIEADAVNIVPITVDSIQIFAGQRYSFVLTADQTPDNYWIRANPNLGS   300

PnLac3   TGFDGGINSAILRY......................................................................................    64
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PsLac    TGFAGGINSAILRYAGATEDDPTTTSSTSTPLLETNLVPLENPGAPGPAVPGGADININPAMAFDFTNFELTINGAPFHAPTAPVLLQILSGATTAASLL   400
PeLac    TGFEGGINSAILRYAGATENDPATTSSTSTPLLETNLVPLENPGAPGPAVPGGADININLAMGFDFTNFEMTINGSPFKAPTAPVLLQILSGATPAASLL   400
PoLac    TGFEGGINSAILRYAGATENDPATTSSTSTPLLETNLVPLENPGAPGPAVPGGADININLAMGFDFTNFEMTINGSPFKAPTAPVLLQILSGATPAASLL   400
PpLac    TGFAGGINSAILRYVGAADADPTTTSSTSTPLLETNLVPLENPGAPGPAVPGGADININLAMAFDFTTFELTINGVPFHPPTAPVLLQILSGASSAASLL   400

PnLac3   ....................................................................................................    64
LsLac    PSGSIYELEANKVVEISMPALAVGGPHPFHLHGHTFDVIRSAGSTTYNFDTPARRDVVNTGTGAN...................................   465
PsLac    PSGSIYELEANKVVEISIPALAVGGPHPFHLHGHTFDVIRSAGSTTYNFDTPARRDVVNTGTGANDNVTIRFVTDNPGPWFLHCHIDWHLEIGLAVVFAE   500
PeLac    PSGSIYALAANKVVEISIPALAVGGPHPFHLHGHTFDVIRSAGSTTYNFDTPARRDVVNTGIDANDNVTIRFVTDNPGPWFLHCHIDWHLEIGLAVVFAE   500
PoLac    PSGSIYALEANKVVEISIPALAVGGPHPFHLHGHTFDVIRSAGSTTYNFDTPVRRDVVNTGIDANDNVTIRFVTDNPGPWFLHC........GLAVVFAE   492
PpLac    PSGSIYELAPNKVVEISMPALAVGGPHPFHLHGHTFDVIRSAGSTTYNFDTPARRDVVNTGTGANDNVTIRFVTDNPGPWFLHCHIDWHLEIGLAVVFAE   500

PnLac3   ...............................                                                                        64
LsLac    ...............................                                                                       465
PsLac    DVTSISAPPAAWDDLCPIYDALSDSDKGGIV                                                                       531
PeLac    DVASIKAPPAAWDDLCPIYDALSDSDRGGIA                                                                       531
PoLac    DVASIKAPPAAWDDLCPIYDALSDSDRGGIA                                                                       523
PpLac    DVTSISAPPAAWDALCPIYDALSDTDKGGII                                                                       531
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(**c**)

Figure S1. Alignments of the amino acid sequences between each isoenzyme of *P. nebrodensis* and their related laccases. Amino acid residues that are conserved in all proteins are shaded *in black*. Residues with conserved substitutions and semiconserved substitutions are indicated with *dark grey* and *light grey*, respectively. (**a**) Alignment of the amino acid sequences between PnLac 1 and some laccases. PnLac1 from *P. nebrodensis* (Lac1); PoLac from *P. ostreatus* (gi: 15594026); PsLac from *P. sapidus* (gi: 67508841); PpLac from *P. pulmonarius* (gi: 61224796); LsLac from *Lentinus sajor-caju* (gi: 32399645); HmLac from *Hypsizygus marmoreus* (gi: 166025441). Type I (T1) Cu binding site and trinuclear Cu binding site are labeled by grey bar at bottom. Domain 1 and domain 2 interfaces of laccases are labeled by black bar at bottom; (**b**) Alignment of the amino acid sequences between PnLac2 and some Pleurotus laccases. PnLac2 from *P. nebrodensis* (Lac2); PoLac from *P. ostreatus* (gi: 291461620); PfLac from *P. florida* (gi: 3005981); PeLacp1 (a laccase precusur) from *P. eryngii* (gi: 78192166); PoLacp (a partial laccase) from *P. ostreatus* (gi: 15594026); PeLacp2 (another laccase precusur) from *P. eryngii* (gi: 56384217); (**c**) Alignment of the amino acid sequences between PnLac 3 and some laccases. PnLac3 from *P. nebrodensis* (Lac3); LsLac from *Lentinus sajor-caju* (gi: 11036962); PsLac from *P. sapidus* (gi: 50724580); PeLac from *P. eryngii* (gi: 56384217); PoLac from *P. ostreatus* (gi: 515019271); PpLac from *P. pulmonarius* (gi: 61224798).