Figure S1

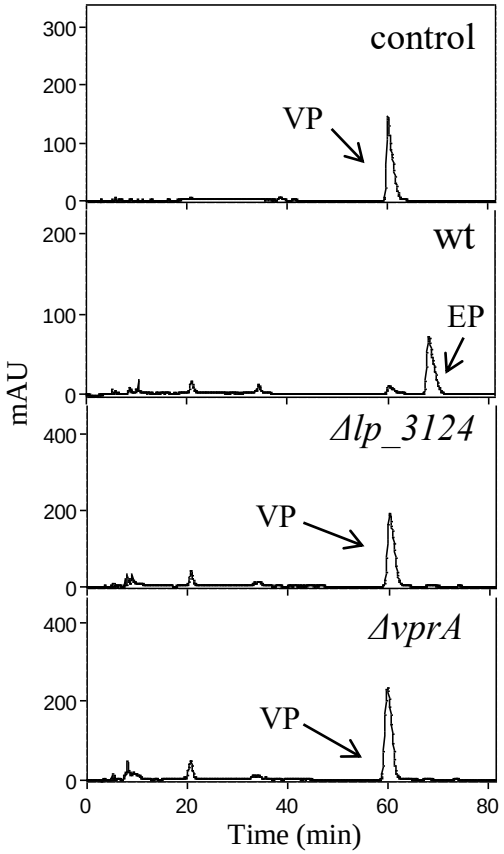

FIG S1 Effect of disruption of *lp_3124* (*vprR*), and *lp_3125* (*vprA*) on vinylphenol reductase activity in *L. plantarum* WCFS1. HPLC chromatograms of *L. plantarum* cultures incubated in 1.5 mM 4-vinylphenol are shown for *L. plantarum* WCFS1 (wild type [wt]), *L. plantarum* WCFS1 (pUCE191-vprR) (Δ*lp_3124* mutant), and *L. plantarum* WCFS1 (pUCE191-vprA) (Δ*lp_3125* mutant). Results for uninoculated medium are also shown (control). The 4-vinylphenol (VP) and 4-ethylphenol (EP) detected are indicated. Chromatograms were recorded at 280 nm.

Figure S2

A

```
VprA      MTLAKHDSYDIVVVGTGAAGTAAALEAAQHGASVLLLEKGRHTGGSSNYTEGLFAVDSYL
DBVR      ------------------------------------------------------------

VprA      QKAQNINVSATDVLKEEVDYSKYRADSRIWRRYLDDSANTVQWLKDQGVEYEGVQAMGAG
DBVR      ------------------------------------------------------------

VprA      EATWHIYKGMGQAVLHDALQPQAQKLGVELLTSTTAITLHQATDGAITGVMIQSAATNET
DBVR      ------------------------------------------------------------

VprA      QVINTAAVILATGGYLNNPDMMQKLTHYDTRRLIPVSSGKGTGDGLRLAWQAGAQQYGTG
DBVR      ------------------------------------------------------------

VprA      MAMLFGGYLKDPSEPSFKYMASQMETAAGQQPLLWLNEHGERFVDEAVVYNFSYAGNALY
DBVR      ------------------------------------------------------------

VprA      TQNQVFSILDQGVINKMAQDGNFMGLGVYVRRGEKMTKLQAE-----------IDAAVAA
DBVR      ----------------------MVKAVAVVRGDSTVKGVVTFEQTSESEPTTIXYNIEG
                                *  .* * **:. .*  .           *   : .

VprA      NKPFIFKANTIEALATKMHLPVDQVTHSIQTYNQYCDNGQDDDFGKNPEYLVKVSQGPFY
DBVR      NDPNALRGFH------------------IHT------------FGDNTNG--CTSAGPHF
         *.*  ::.                   *:*            **.* :    .* **.:

VprA      -GFELNVGA-------FCTMGGLKVTTNN--------EVLDTTGQ-PIT---GLYAAGND
DBVR      NPFGKTHGAPTDENRHVGDLGNIKTDANGVAKGTIKDKLVKLIGXNSIIGRTVVVHAGTD
           *  . **       .  :*.:*. :*.        :::.  *   *     :  **.*

VprA      AAGLTGDTYGPNMPGTCVGYAFYSGRNSGRHAAQYTHQQSIVSH
BDVR      DLGKGGDAG-S----------LQTGNAGGRPACGVIGLSA----
           *  **:             : :*. .** *.     .:
```

B

```
VprA      ------------------------------------------------------------
HcrB      MKFVGIVGTNAQHSYNRMLLEFMQRHFATQAEIEILELTDVPMFDESNDQTDSTIIQNFA

VprA      ------------------------------------------------------------
HcrB      TKIATADGVIIASPEHNHSVPSALKSIIEWLSFKIHPLDGQAVMIVGASYSVQGSSRAQL

VprA      ------------------------------------------------------------
HcrB      HLRQILDAPGVNASVMPGSEFLLGRAQTAFDDQGNLKVQGTVDFLDSCFAKFQKFATIVA
```

```
VprA      ------------------------------------------------------------
HcrB      EMRAPEALSFAPGTYQVTATGHNGELPMRVTLSADRIENIEIDTSSETQGIADVAFERIP


VprA      ------------------------------------------------------MTLAKHDS
HcrB      KEIIAGQTLAVDAISGASITSHGVIDGVARAVKEAGANPDDLKKRRATKQVAQPAVKEVT
                                                                  .*. :

VprA      YDIVVVGTGAAGTAAALEAAQHGASVLLLEKGRHTGGSSNYTEGLFA-------------
HcrB      TDVVVVGAGGAGMTAAAKVLQAGHQAVVLEKFPAVGGNTVRAGGPMNAADPDWQRQFAAL
           *:****:*.** :** :. * * ..::***   .**.:  : * :

VprA      ---------------------------------VDSYLQKAQNINVS--ATDVLK------
HcrB      PGEKQTLKDLSERDESTIAPEYRADFRKLKQQIDAYLTANTNQKGTLFDSTLLHRIQTYL
                                           :*:**    * : :   : :*:

VprA      ----EEVDYSKYRADSRIWRRYLDDSANTVQWLKDQGVEYEGVQAMGAGEATWHIYK-GM
HcrB      GGQRTDLNGQEIHGQYDLVKELTDNALDSVKWLQSIGVKFDESQVTMPVGAIWRRGHKPM
               ::: .: :.:  : :.  *:: ::*:**:. **:::  *.     * *:  :  *

VprA      GQAVL--HDALQPQAQKLGVELLTSTTAITLHQATDGAITGVMIQSAATNETQVINTAAV
HcrB      GDLGFAYIKTLRAFVEQQGGTIMTE-TPVKELLVTDGQVRGVIATN-AAHEKVIVHADAV
          *:  :   .:*:  .:: *  ::*. * :.   .*** : **:  . *::*. :::: **

VprA      ILATGGYLNNPDMMQKLTHYDTRR---LIPVSSGKGTGDGLRLAWQAGAQQYGTGMAMLF
HcrB      ILASGGFAANTKMLQKYNTYWTAIDDDVKTTNSPAMTGDGIRLGTSVGAALVGMGFSQMM
          ***:**:  * .*:** . * *     :  ..*   ****:**. ..**   * *:: ::

VprA      GGYLKDPSEPSFKYMASQMETAAGQQPLLWLNEHGERFVDEAVVYNFSYAGNALYTQNQV
HcrB      PV-----SDPETGELFSGLQ--VPPANFVMVNQQGKRFVNEYGSRDEL--TQAAIDNGSL
                 *:*.   : * ::  .    :: :*::*:***:*    :     :*   :..:

VprA      FSILDQGVINKMAQDGNFMGLGVYVRRGEKMTKLQAEIDAAVAANKPFIFKANTIEALAT
HcrB      FYLIADDEIKKT-----------------AYNTTQAKIDQQVA--NGTLFRADTLTDLAQ
          * :: :. *:*                    .. **:**  **  :  :*:*:*:  **

VprA      KMHLPVDQVTHSIQTYNQYCDNGQDDDFGKNPEYLVKVSQGPFYGFELNVGAFCTMGGLK
HcrB      QIGMDPAALTKTIADYNRYVDAGEDPEFHKT-AFDLKVAVAPFYATPRKPATHHTMGGLK
          :: :    :*::*  **:* * *:* :* *.  : :**: .***.   : .:. ******

VprA      VTTNNEVLDTTGQPITGLYAAGNDAAGLTGDTYGPNMPGTCVGYAFYSGRNSGRHAAQYT
HcrB      IDSDAHVLNTDGQVIDGLYAAGEVAGGIHAG---NRLGGNSLSDIFTFGRIAAAHAVAEH
          : :: .**:* ** * ******: *.*: ..    .: *..:.  *  ** :. **.

VprA      HQQSIVSH
HcrB      -VDPVTA-
            : :.:
```

FIG S2 Comparison of amino acid sequences of *L. plantarum* WCFS1 VprA protein and vinylphenol reductase from *D. bruxellensis* (DBVR, I2JWC1) (A) or to *L. plantarum* WCFS1 hydroxycinnamate reductase (HcrB) (B). Multiple alignments were done using the programs ClustalOmega after retrieval of sequences from BLAST homology searches. Residues that are identical (*), conserved (:) or semiconserved (.) in all sequences are indicated. Dashes indicated gaps introduced to maximize similarities. The Rossmann-fold GXGXXG motif is highlighted in green.

Figure S3

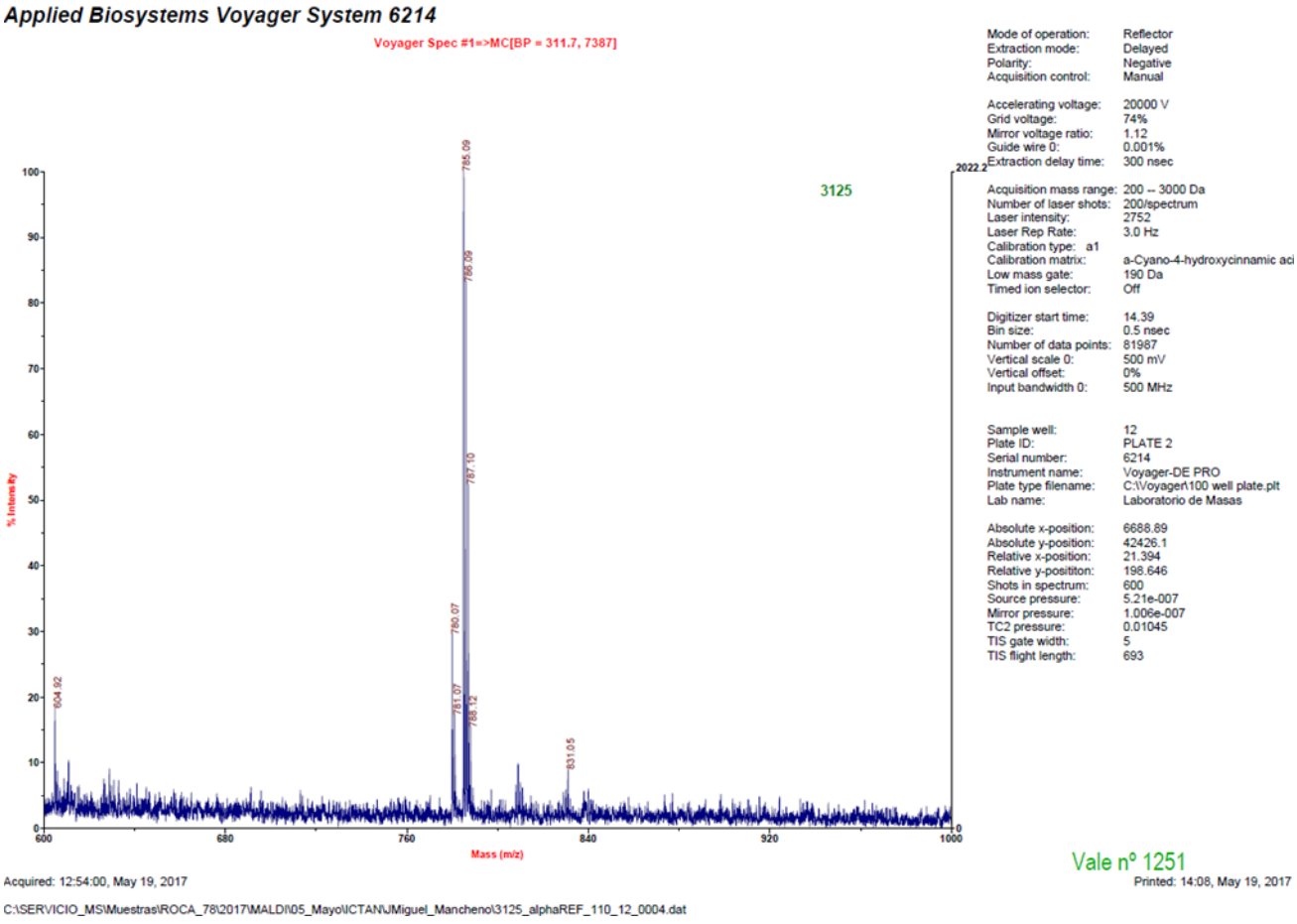

FIG S3. Mass spectra of FAD. The yellow VprA protein was subjected to mass spectrometry analysis and the FAD flavin cofactor (785.09 Da) was identified.

Figure S4

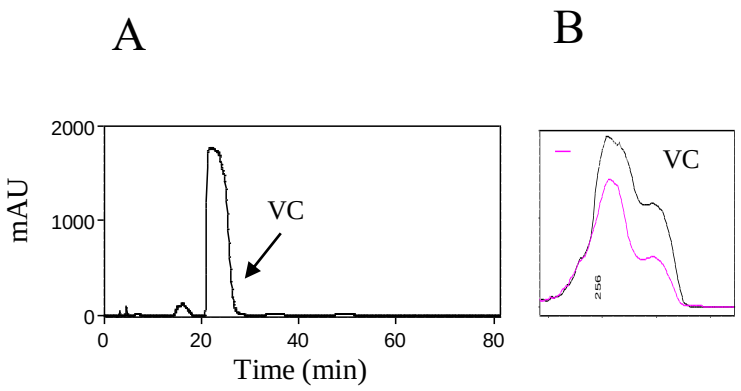

FIG S4 Production of 4-vinylcatechol from *p*-coumaric acid. (A) Chromatograms of supernatants from *E. coli* cells bearing pURI3-pdc plasmid overexpressing the *pdc* gene from *L. plantarum* (Rodríguez et al., 2008. J Agric Food Chem 56:3068) grown in the presence of caffeic acid. Chromatogram was recorded at 280 nm. (B) Comparison between spectra of the compound produced and 4-vinylcatechol identified previously by LC-DAD/ESI-MS (Rodríguez et al., 2008. J Agric Food Chem 56:3068). The same *E.coli* strain was used previously to produce vinyl derivatives from alkaline hydrolysates of corn cobs (Salgado et al., 2012. Bioresource Technol 117:274-285; Salgado et al., 2014. Enz Microb Technol 58-59, 22-28).

Figure S5

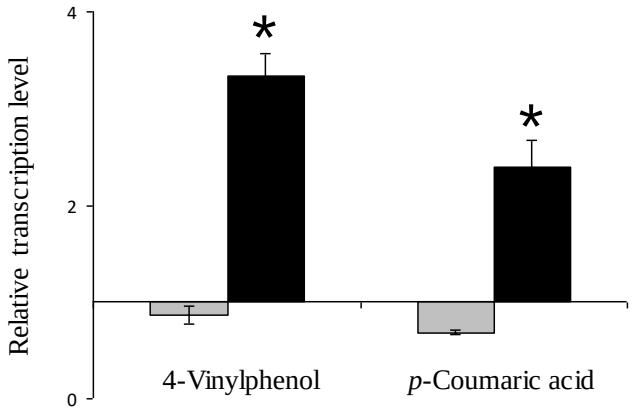

FIG S5 Relative transcriptional expression of *vpr* genes in *L. plantarum* WCFS1 in response to the presence of two hydroxycinnamic derivatives, 4-vinylphenol and *p*-coumaric acid. *L. plantarum* cultures were exposed during 10 min at 1.5 mM of 4-vinylphenol or *p*-coumaric acid. Expression levels were calculated with the 7500 Fast System relative quantification software using *L. plantarum ldh* gene as endogenous gene and the growth in the absence of phenolic compound as growth condition calibrator. Expression level of *vprR* and *vprA* genes are represented by grey or black colour bars, respectively. The experiments were done in triplicate. The mean value and the standard error are shown. Asteriks indicate a *p* value <0.1.

Figure S6


1657
LFA --MWPKAKQYDLVIVGGGLSGLVAAVSAREQQLSTLVLEKGRSIGGDGNYVEGAMGVDSY
LCO --MFPKKTDYDLVIVGGGLSGLAAAVAAGEKKLATLVLEKGRTLGGDGNYVEGAMGVDST
LRO MPNITRKDAYDVVVVGTGAAGTSAALTAAQNGASVLLLEKGRHTGGSSNYTEGLFAVDSY
LBI -MTISQKERYDIVVVGTGAAGTSAALEATQQGATVLLLEKGRHTGGSSNYTEGLFAVDSY
LSI -MSFTKQERYDVVVVGTGAAGTSAALEAAQHGASVLLLEKGRHTGGSSNYTEGLFAVDSY
LPC -MSISQKTQYDVVVVGTGAAGTSAALEAAQHGASVLLLENGRHTGGSSNYTEGLFAVDSY
LPL -MTLAKHDSYDIVVVGTGAAGTAAALEAAQHGASVLLLEKGRHTGGSSNYTEGLFAVDSY
LPE -MTLTKRTSYDIVVVGTGAAGTAAALEAAQHGATVLLLEKGRHTGGSSNYTEGLFAVDSY
LPA -MTLTEHTSYDIVVVGTGAAGTAAALEAAQHGATVLLLEKGRHTGGSSNYTEGLFAVDSY
LFA LQKAAGIDINPTELLQDELAYSHYEANAPHLKKYIDASGGVIDWLHNLGVKFAKVGPQGK
LCO MQKDAGISIDKTELLQDELTYSHYEASAPHLKKLIDASGATIDWLQSLGVTFTKVGPQGK
LRO LQKQQGITVSGTDVLKEEVAYSKYRADSRIWRNYVDDSADTVKWLNEQGVEYEGVQAMGD
LBI LQKQQGIQVSGTDVLKEEVEYSKFKADSRIWRRYVDDSANTVQWLRDQGVTYEGVQAMGA
LSI LQKEQGIKVSGTDVLKEEVEYSKYKADSRIWRNYVDDSANTVQWLKDQGVKYEGVQAMGA
LPC LQKAQGIKVSATDVLREEVDYSKYRADSRIWRNYLDDSTGTVQWLKDQGVKYEGVQAMGA
LPL LQKAQNINVSATDVLKEEVDYSKYRADSRIWRRYLDDSANTVQWLKDQGVEYEGVQAMGA
LPE LQQAQHIHVSATEVLKEEVDYSKYRADSRIWRQYLDDSADTVQWLKDQGVEYEGVQAMGA
LPA LQQAQNIHVSATDVLKEEVDYSKYRADSRIWRQYLDDSANTVQWLKDQGVEYEGVQAMGA
LFA SWSTIHTFAGGGHAAVQ-TLYANATQAGAEFVTSVSAQRLQQ-HDGHVTGVVIMDENTGQ
LCO SWPTIHTFEGGGHAAIE-KLKAKADEYGVEFATSVSAQKLLQ-ANGHISGLAVKNEATGQ
LRO GEATWHIYKGMGNAVINDALIPQAKKLGVEVLTSTAATNIDQ-ENGKISRVTIKNLANNL
LBI GEATWHIYDGMGQSVLHDHLLPQAEKLGVELLTSTTAVELLQDQTGAITGVKIQDEITRD
LSI GEATWHIYEGMGQSVLHDALLPQAEKLGVEVRTLTTAIALHQDADGAINGVTIRDEASQE
LPC GEATWHIYEGMGQSVLHDALQPQAEKLGVELLSSTTVTALHQNTDGAINGLTIQDEATGT
LPL GEATWHIYKGMGQAVLHDALQPQAQKLGVELLTSTTAITLHQATDGAITGVMIQSAATNE
LPE GEATWHIYKGMGQAVLHDALQPKALALGVELLTSTTAIDLHQADNGTITGVTIQNAATKE
LPA GEATWHIYKGMGQAVLHDALQPKAQSLGVEMLTSTTVTALHQADDGTITGVTIQDAGTKE
LFA THEIAAKNVLLATGGYVDNPDLVKTRVA-DNHRLMAVSDGKSTGDGMQLAWQVGAKHYSM
LCO YRDLRAQNVLLATGGYVDNPELIKERTP-FVGRLLTVSDGKSTGDGMQLAWAAGAQHYSM
LRO SESVKTSSVILATGGYLNNAEMMSKMTHYDTSRLVTVSSGKGTGDGLQMAWNLGARKYGT
LBI LQNVQTSAVILATGGYLNNPEMMAQLTAYDTSRLVPVSSGKGTGDGLRLAWNAGAKQYGT
LSI TQDVATGAVVLAAGGYLNNPDMMAKLTQYDTSRLIPVSSGKGTGDGLQLGWKAGAKQYGT
LPC TEDIKAGAVVLATGGYLNNPEMMKELTHYDTSRLIQVSSGKGTGDGLRLAWKIGAKKYGT
LPL TQVINTAAVILATGGYLNNPDMMQKLTHYDTRRLIPVSSGKGTGDGLRLAWQAGAQQYGT
LPE TQLVNTHAVILATGGYLNNPEMMQNLTHYDTSRLIPVSSGKGTGDGLRLAWQAGAQQYGT
LPA TQAIATSAVILATGGYLNNPEMMASLTHYDTRRLIPVSSGKGTGDGLQLAWDAGAQKYGT
LFA GAIQYGGGAIYDKTQPPFVHMASQLAAAATQEAILWVNERGERFVNEDVNDNMCHAGSAI
LCO GAIQYGGGAIYDRQTPPFVHMMSQLGVVATQEAILWVNERGERFVNEDVNDNMCHAGGAI
LRO GTAMLFGGYLKDPSVPAFKFMSSEMNTAAGQQPLLWVNENGERFVDEAVVYNFSFAGNAL
LBI GMAMLFGGYLKDPDEPSFKLMASQMNTAAGQQPLLWVNEHGERFVDESVVYNFSYAGNAL
LSI GMAMLFGGYLKDPDEPSFKFMASQMNTAAGQQPLLWVNEHGERFVDESVVYNFSYAGNAL
LPC GMAMLFGGYLKDPDEPSFKYMASQMETAAGQQPLLWLNEHGERFVDEAVVYNFSYAGNAL
LPL GMAMLFGGYLKDPSEPSFKYMASQMETAAGQQPLLWLNEHGERFVDEAVVYNFSYAGNAL
LPE GMAMLFGGYLKDPSEPSFKYMASQMETAAGQQPLLWLNEHGERFVDEAVVYNFSYAGNAL
LPA GMAMLFGGYLKDPAEPSFKYMASQMETAAGQQPLLWLNEHGERFVDEAVVYNFSYAGNAL
LFA LTQARTFSILDQAAVDHLTNVGLYKEVGNSPISPEKFDQLTSEIDDTLKQHAKYLTSAAT
LCO LTQSRVFSIFDQAAVDHLANVGLFKEVGNSPVSPDKLDKLPGEIETDLGKHASYLTKADS
LRO YTQNKVFSILDTAVIDEMAKSGNFMGLGVYVKRGQKMTKLKDEIDEAVAEERPFIFKADT
LBI YTQNQVFSILDQGVIDKMATDGHFMGLGVYVKRGEKMATLQAELDETVAAHKPFIFKADT
LSI YTQNQVYSILDQGVIDKMAKDGNFMGLGVYVKRGEKMDKLQAEIDGAVAADKPFIFKADT
LPC YTQNKVFSVLDQGVIDKMAKDGNFMGLGVYVRRGEKMVKLQAELDAAVSANKPFIFKADT
LPL YTQNQVFSILDQGVINKMAQDGNFMGLGVYVRRGEKMTKLQAEIDAAVAANKPFIFKANT
LPE YTQNQVFSILDQGVIDRMARDGNFMGLGVYVRRGEKMTKLQAEIDAAVAANKPFIFKAKT
LPA YTQNQVFSILDQGVIDRMAQDGNFMGLGVYVRRGEKMTKLQSEIDAAVTAKKPFIFKADT

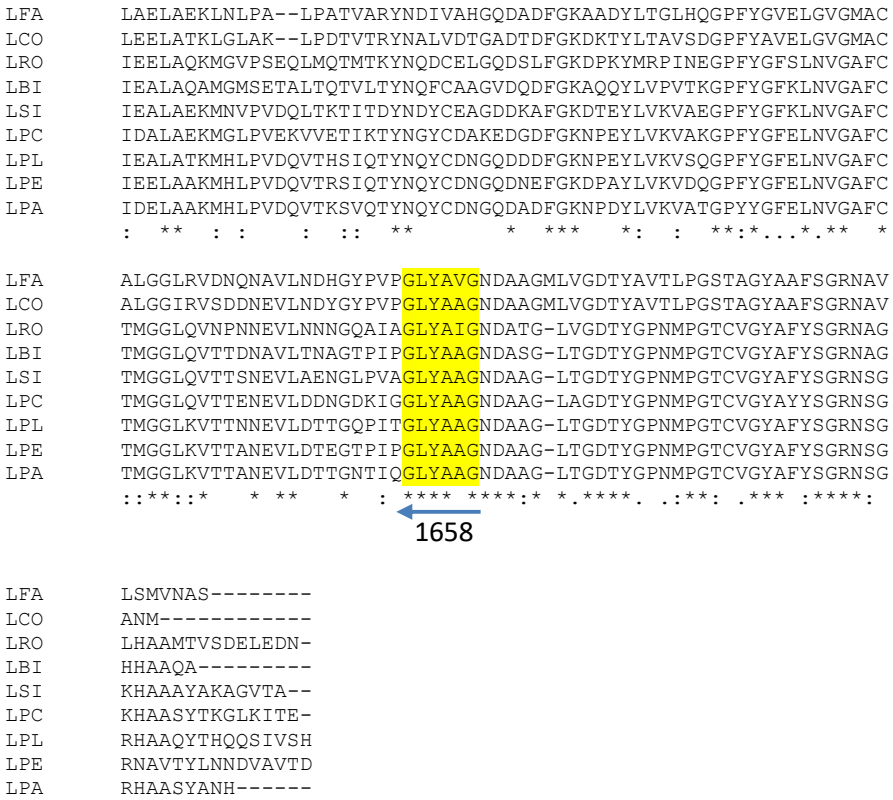

FIG S6 Comparison of amino acid sequences of putative VprA proteins from lactic acid bacteria. Multiple alignments were done using the program ClustalOmega after retrieval of sequences from BLAST homology searches. The VprA proteins are from *Lactobacillus plantarum* WCFS1 (LPL) (lp_3125), *Lactobacillus paraplantarum* DSM 10667 (LPA) (A0A0R1RBK3), *Lactobacillus pentosus* DSM 10667 (LPE) (A0A0R1FK87), *Lactobacillus fabifermentans* DSM 21115 (LFA) (A0A0R2NSN6), *Lactobacillus collinoides* DSM 20515 (LCO) (A0A0R2BBL2), *Lactobacillus paracollinoides* DSM 15502 (LPC) (A0A0R1T9H1), *Lactobacillus bifermentans* DSM 20003 (LBI) (A0A0R16M13), *Lactobacillus similis* DSM 23365 (LSI) (A0A0R2F9E3), and *Lactobacillus rossiae* DSM 15814 (LRO) (A0A0R1RHT0). Residues that are identical (*), conserved (:) or semiconserved (.) in all sequences are indicated. Dashes indicated gaps introduced to maximize similarities. The Rossmann-fold GXGXXG motif is highlighted in green. Degenerate primers were designed on the conserved domains yellow highlighted.