

Supplementary Material

P40 and P75 are singular functional muramidases typically found in the *Lactobacillus casei/paracasei/rhamnosus* taxon

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1. Supplementary Figure Legends

Figure S1.- Translation of the *cmuA* gene encoding protein P40. Sequences encoding domains CwlO (COG3883) and Nlpc/P60 are shadowed and indicated. Putative active triad amino acid residues C-H-N tentatively deduced from the consensus described by Aramini *et al.* (Aramini *et al.*, 2008) are marked in bold and underlined.

Figure S2.- Alignment of *cmuB* gene and *mSPa* genes encoding P75 in *L.casei* BL23 and *L.rhamnosus* GG. Shadowed sequences indicate the location of Tandem Repeats in *L.casei* BL23.

Figure S3.- Complete PAGE picture of Figure 3.

Figure S4.- 3D *in silico* modeling of the structure of P40 and P75. The structural models were built using RaptorX2 (<http://raptorx2.uchicago.edu/StructurePropertyPred/predict/>) (Källberg *et al.*, 2012; Wang *et al.*, 2016). Unfortunately, no 3D structures have been determined of homologous proteins that would allow fully reliable structural predictions. We selected the RaptorX2 model because for P40 there was a moderately satisfactory prediction based on PDB template 4cgkA corresponding to the CHAP domain-containing protein of *Streptococcus pneumoniae* R6 [WP_000727012.1]. fGlobal distance test (GDT) was 49% and P-value 1.9×10^{-5} , that suggest at least a good partial prediction. SWISS-MODEL (<https://swissmodel.expasy.org/>) (Waterhouse *et al.*, 2018) was also used for P40 using PcsB from *Streptococcus pneumoniae* and RipA from *Mycobacterium tuberculosis* as templates (PDB nos. 4cgk.1.A and 6ewy.1.A) (35% and 28% similarity) and the general configuration was similar to that found with RaptorX2. In the case of P75, the highest P-value and GDT were found with templates 3pvqA (p= 2.6×10^9 , GDT= 38%), 6bimA (p= 3.4×10^9 , GDT=

36%) and 3h41A ($p= 2.8 \times 10^8$, GDT= 35%), which are respectively the NlpC/P60 family protein of *Bacteroides thetaiotaomicron* [WP_011107705.1], C40-NlpC/P60 superfamily cysteine peptidase from *Trichomonas vaginalis* G3 [XP_001276902.1] and the NlpC/P60 family protein of *Bacillus cereus* [WP_061182922.1]. This could be interpreted as suboptimal structural predictions, that again are due to the lack of more similar references.

Figure S5.- Lytic activity assay of P40 and P75 against *L.casei*, *E. faecalis*, *L. monocytogenes* and *S. aureus*. (A) pH optimization. (B) Effect of the proteins and Triton after 2 hours of incubation at the selected pH.

Figure S6.- Western blot obtained using anti-P40 polyclonal antibodies isolated from purified native P40 (Yan et al., 2007).

2. Supplementary Tables

3. **Table S1.-** List of putative proteins containing C-terminal NlpC/P60 domain in the annotated genomes of representative strains of *Lactobacillus* and *Lactococcus*, as an illustration of their abundance.

Species_Strain	Ref. Genome annotation	Accession numbers - Annotated function
<i>Lactobacillus casei</i> BL23	(Maze et al., 2010)	WP_003572828.1 - CHAP domain-containing protein[P40] WP_012490875.1 - NlpC/P60 family protein[P75] WP_012491782.1 - hypothetical protein WP_012491062.1 - hydrolase
<i>Lactobacillus rhamnosus</i> GG	(Kankainen et al., 2009)	AXI93082.1 CHAP - domain-containing protein[P40] AXI93342.1- glycoside hydrolase [P75] AXI94896.1 - hypothetical protein DU507_10550
<i>Lactococcus lactis subsp cremoris</i> MG1363	(Wegmann et al., 2007;Linares et al., 2010)	AAA25230- secreted protein CAL97110 - conserved hypothetical protein CAC93672 - gamma-glutamyl-diamino acid-endopeptidase
<i>Lactobacillus plantarum</i> WCFS1	(Siezen et al., 2012)	WP_060677571.1 - NlpC/P60 family protein WP_011101812.1 - NlpC/P60 family protein WP_011101674.1- peptidoglycan endopeptidase WP_011102154.1 - LysM peptidoglycan-binding domain-containing protein WP_011101331.1 - gamma-D-glutamate-meso-diaminopimelate muropeptidase
<i>Lactobacillus delbrueckii subsp. bulgaricus</i> ATCC BAA-365	(van de Guchte et al., 2006)	ABJ59268.1 -Cell wall-associated hydrolase ABJ57819.1 - Cell wall-associated hydrolase ABJ59266.1 - Cell wall-associated hydrolase ABJ59129.1 - dipeptidyl-peptidase VI, Cysteine peptidase, MEROPS family C40 ABJ59265.1 - Cell wall-associated hydrolase
<i>Lactobacillus brevis</i> ATCC 367	(Makarova et al., 2006)	ABJ64517.1 - Cell wall-associated hydrolase ABJ63531.1 - D-alanyl-D-alanine carboxypeptidase ABJ64515.1 - Cell wall-associated hydrolase
<i>Lactobacillus gasseri</i> ATCC 33323	(Makarova et al., 2006)	ABJ60987.1 - Cell wall-associated hydrolase

Table S2.- FASTA sequences of BL23 *cmuB* gene and *L. paracasei* variants of minisatellites.

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Lactobacillus paracasei strain Zhang, complete genome

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[CDS](#)

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Tables S3.- Sequences used in the phylogenetic analysis.

Table S3.1.- P40 homologous sequences used for phylogenetic reconstructions.

Strain	Acc. N°
<i>Lactobacillus casei</i> BL23	CAQ65155
<i>Lactobacillus paracasei</i> DUP 13076	WP_101869740
<i>Lactobacillus paracasei</i> L9D	WP_071252149
<i>Lactobacillus paracasei</i> subsp. <i>paracasei</i> Lpp221	WP_016379272
<i>Lactobacillus casei</i> subsp. <i>casei</i> ATCC 393	WP_025013825
<i>Lactobacillus rhamnosus</i> HN001	WP_005687968
<i>Lactobacillus rhamnosus</i> GG	WP_014568889
<i>Lactobacillus zeae</i> DSM 20178	WP_010490240
<i>Lactobacillus sakei</i> subsp. <i>sakei</i> 23K 1	WP_011374356
<i>Lactobacillus sakei</i> subsp. <i>sakei</i> 23K 2	WP_011373952
<i>Lactobacillus murinus</i> DSM 20452	WP_004051788
<i>Lactobacillus animalis</i> DSM 20602	KRM59177
<i>Lactobacillus curvatus</i> MRS6	WP_089557112
<i>Lactobacillus graminis</i> DSM 20719	WP_057908021
<i>Lactobacillus fuchuensis</i> DSM 14340	WP_056950269
<i>Lactobacillus manihotivorans</i> DSM 13343	WP_056964416
<i>Lactobacillus nasuensis</i> JCM 17158	WP_054723415
<i>Enterococcus asini</i> ATCC 700915	WP_010752994
<i>Lactococcus lactis</i> subsp. <i>lactis</i> K337	WP_058204561
<i>Lactococcus piscium</i> DSM 6634	WP_096813813
<i>Streptococcus equi</i> subsp. <i>ruminatorum</i> CECT 5772	WP_037582442
<i>Streptococcus pneumoniae</i> R6	WP_000727012
<i>Streptococcus sanguinis</i> 2908	CEL91468
<i>Vagococcus teuberi</i> DSM 21459	WP_071456026

Table S3.2.- P75 homologous sequences used for phylogenetic reconstructions.

Strain	Acc. N°
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<i>Lactobacillus paracasei</i> lpp122 1	WP_016383801
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<i>Lactobacillus rhamnosus</i> GG	WP_005687638

<i>Lactobacillus casei</i> subsp. <i>casei</i> ATCC 393	WP_025013844
<i>Lactobacillus casei</i> LC5	WP_087911410
<i>Lactobacillus zaeae</i> DSM 20178	WP_010492712
<i>Lactobacillus nasuensis</i> JCM 17158	WP_054723265
<i>Lactobacillus cameliae</i> DSM 22697	WP_056989569
<i>Lactobacillus fuchuensis</i> DSM 14340	WP_056950327
<i>Lactobacillus sakei</i> subsp. <i>sakei</i> 23K	WP_011374023
<i>Lactobacillus sakei</i> FAM18311	WP_076632543
<i>Lactobacillus curvatus</i> FBA2	WP_064777492
<i>Lactobacillus graminis</i> DSM 20719	WP_057907367
<i>Lactobacillus amylophilus</i> DSM 20533	WP_056947108
<i>Lactobacillus helsingborgensis</i> Bma 5	WP_046327598
<i>Lactobacillus manihotivorans</i> DSM 13343	WP_082611783
<i>Lactobacillus perolens</i> DSM 12744	WP_083487735
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<i>Lactobacillus harbinensis</i> DSM 16991	WP_027829605
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<i>Lactobacillus farciminis</i> DSM 111452	WP_059074409
<i>Lactobacillus farciminis</i> DSM 20184	WP_010020650
<i>Lactobacillus futsaii</i> JCM 17355	WP_057811975
<i>Lactobacillus kimchiensis</i> DSM 24716	WP_057881517
<i>Lactobacillus versmoldensis</i> DSM 14857	WP_010624150
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<i>Lactobacillus nodensis</i> DSM 19682	WP_056979871
<i>Lactobacillus nantensis</i> DSM 16982	WP_057891330
<i>Lactobacillus heilongjiangensis</i> DSM 28069	WP_041499677

Table S4.- Blast alignment of *L.casei/paracasei/rhamnosus* genes encoding P40 proteins for the selection of PCR primers

Database: Nucleotide collection (nt)
36,527,618 sequences; 117,866,738,766 total letters
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strain BL23
Length=1236

Sequences producing significant alignments:	Score (Bits)	E Value
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gb CP002616.1 <i>Lactobacillus casei</i> LC2W, complete genome	2283	0.0
emb FM177140.1 <i>Lactobacillus casei</i> BL23 complete genome, str...	2283	0.0
gb CP006690.1 <i>Lactobacillus casei</i> 12A, complete genome	2067	0.0
gb CP001084.2 <i>Lactobacillus casei</i> str. Zhang, complete genome	2061	0.0
gb CP005486.1 <i>Lactobacillus casei</i> LOCK919, complete genome	2061	0.0
gb CP005484.1 <i>Lactobacillus rhamnosus</i> LOCK900, complete genome	900	0.0
dbj AP012544.1 <i>Lactobacillus casei</i> subsp. <i>casei</i> ATCC 393 DNA...	889	0.0
dbj AP011548.1 <i>Lactobacillus rhamnosus</i> ATCC 53103 DNA, compl...	883	0.0
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(BamHI)
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For the L.rhamnosus sequences, see at the end full sequences

RhamP40N for 5'- GTTGGATCCGACACAAGTGCCAG (BamHI)

RhamP40N rev 5'-GTAGGGCCCTTATTCTTTGTTCAAAGC (SmaI)

GCTTTGAACAAAGAA

RhamP40C for 5'- GTTGGATCCGCTGATCACAAAGAC

(BamHI)

RhamP40C rev 5'-GTAGGGCCCTTACCGGTGGATGTAA (SmaI)

TTACATCCACCGGT

ALIGNMENTS

Query	1	ATGAAATTCAATAAAGTCATGATCACGTTGGTTGCTGCAGTTACCTTAGCAGGTTCTGCT	60
HE970764	23408	23349
CP002618	23409	23350
CP002616	23409	23350
FM177140	23411	23352
CP006690	22250	22191
CP001084	22759	22700
CP005486	20930	20871
CP005484	25290CA.....G..A.....A.....G.....T.	25231
AP012544	20379CT.....T..A.....C.....C.....C	20438
AP011548	31251CA.....G..A.....A.....G.....T.	31192
FM179322	31251CA.....G..A.....A.....G.....T.	31192

Query	61	AGCGCCGTAACACCAGTTTTTCGCTGATACAAGCGACAGTATCGCTTCCAACAAAAGCGAA	120
HE970764	23348	23289
CP002618	23349	23290
CP002616	23349	23290
FM177140	23351	23292
CP006690	22190	22131
CP001084	22699	22640
CP005486	20870	20811
CP005484	25230G.....C.....T.C...C.....A..T....G.....	25171
AP012544	20439G.....C.....T.C...C.....T.....T.....	20498
AP011548	31191T.....G.....C.....T.C...C.....A..T....G.....	31132
FM179322	31191T.....G.....C.....T.C...C.....A..T....G.....	31132

Query	121	ACCAATGCACTGTTGAAGCAAATTGAAGATGCGAACACAGAAGTTATCAATTTGAACAAG	180
HE970764	23288	23229
CP002618	23289	23230
CP002616	23289	23230
FM177140	23291	23232
CP006690	22130	22071
CP001084	22639	22580
CP005486	20810	20751
CP005484	25170C.ATT.A..A.....C.....CA..T.....T.....G.....CC.C.....	25111
AP012544	20499	..G..C.ATT.A..A..A.....C.....CA..C.....T..G..C.....C.T.....A	20558
AP011548	31131C.ATT.A..A.....C.....CA..T.....T.....G.....CC.C.....A	31072
FM179322	31131C.ATT.A..A.....C.....CA..T.....T.....G.....CC.C.....A	31072

Query	181	CAGATCGATACCAAGAATGGTCAAATCAGCGATGCAACGGCTAAGATCAGCGCAACTGAT	240
HE970764	23228	23169
CP002618	23229	23170
CP002616	23229	23170
FM177140	23231	23172
CP006690	22070	22011
CP001084	22579	22520
CP005486	20750	20691
CP005484	25110T...G.T.....CG.....T..C..T..T..C.....T.....G...	25051
AP012544	20559T..CGAT.....TCG.....T.....C..A..C.....T.....C...	20618
AP011548	31071T...G.T.....CG.....T..C..C..T..C.....T.....G...	31012
FM179322	31071T...G.T.....CG.....T..C..C..T..C.....T.....G...	31012

Query	241	GCCAAGATTTCACTACTCAGCGGTGAAATTTGTTGCCGCTCAAAGAATGTCACAGCACGC	300
HE970764	23168	23109
CP002618	23169	23110
CP002616	23169	23110
FM177140	23171	23112
CP006690	22010	21951
CP001084	22519	22460
CP005486	20690	20631
CP005484	25050CG...GT.G..T..C.....ACC..T.....C...G...C..G	24991

AP012544	20619	...C....CAA....T.G....CC....CACG..T..C.....C..G..G.....G	20678
AP011548	31011CG....GT.G..T..C....CACC..T.....C...G...C..G	30952
FM179322	31011CG....GT.G..T..C....CACC..T.....C...G...C..G	30952
Query	301	AAGAACAACCTGAAAAGATCAGTTGATCTCCTTGCaaaaaaGGCTGGCAACTCTGTCAGC	360
HE970764	23108	23049
CP002618	23109	23050
CP002616	23109	23050
FM177140	23111	23052
CP006690	21950	..A.....	21891
CP001084	22459	..A.....	22400
CP005486	20630	..A.....	20571
CP005484	24990G....A....T...C.T....G..A..C...GT..A..T...	24931
AP012544	20679C..AC...T...C.T....G....G...G..T...	20738
AP011548	30951G....A....T...C.T....G..A..C...GT..A..T...	30892
FM179322	30951G....A....T...C.T....G..A..C...GT..A..T...	30892
Query	361	GGCAATGTCTATATCGACTTTGTTCTGAATTCAGAA-CT-TGTCTGACTTAATTGCC	418
HE970764	23048-.-.....	22991
CP002618	23049-.-.....	22992
CP002616	23049-.-.....	22992
FM177140	23051-.-.....	22994
CP006690	21890-.-.....	21833
CP001084	22399-.-.....	22342
CP005486	20570-.-.....	20513
CP005484	24930T..T..C..GT...C..A...-G.C.A.....G.....	24873
AP012544	20739C.....C..GT...C..A..A.GT.-.-..G....G..C....	20796
AP011548	30891T..T..C..GT...C..A...-G.C.A.....G.....	30834
FM179322	30891T..T..C..GT...C..A...-G.C.A.....G.....	30834
Query	419	GCACCATGACGGTTGGCAAACCTTAGCCAAGCCAGCAAAGATGCGC-TT-GATGCCGTGAC	476
HE970764	22990-.-.....	22933
CP002618	22991-.-.....	22934
CP002616	22991-.-.....	22934
FM177140	22993-.-.....	22936
CP006690	21832-.-.....	21775
CP001084	22341-.-.....	22284
CP005486	20512-.-.....	20455
CP005484	24872	..T.....A..C....GT.A..T.....-.-..T..G....G..A..	24815
AP012544	20797C.....C.....G..A...-G-..C..T....	20854
AP011548	30833	..T.....A..C....GT.A..T.....-.-..T..G....G..A..	30776
FM179322	30833	..T.....A..C....GT.A..T.....-.-..T..G....G..A..	30776
Query	477	AGTTGCCAAGGATAAATTAGCTGGCTTGAAGACGGAACAGGAAAATGCCCGTCAGACGCT	536
HE970764	22932	22873
CP002618	22933	22874
CP002616	22933	22874
FM177140	22935	22876
CP006690	21774	21715
CP001084	22283	22224
CP005486	20454	20395
CP005484	24814	C..C....A....G....C..CT.....GT.....CG..A.....CT.	24755
AP012544	20855	C.....C...C..G..A..AC....ACC.....CA..G.....T.	20914
AP011548	30775	C..C....A....G....C..CT.....GT.....CG..A.....CT.	30716
FM179322	30775	C..C....A....G....C..CT.....GT.....CG..A.....CT.	30716
Query	537	GGTTTCAACTAAGGC-T-TCATTGGAAACCCAGAAATCACAGTTGGTTTCCCTACAAAA	594
HE970764	22872-.-.....	22815
CP002618	22873-.-.....	22816
CP002616	22873-.-.....	22816
FM177140	22875-.-.....	22818
CP006690	21714-.-.....	21657
CP001084	22223-.-.....	22166
CP005486	20394-.-.....	20337
CP005484	24754G....A..A.C...-.-...G..A..G....C.A.AGA...T....-	24698
AP012544	20915C...T..-A-..GC.T.....G..T.....A..T.G....-	20971
AP011548	30715G....A..A.C...-.-...G..A..G....C.A.AGA...T....-	30659
FM179322	30715G....A..A.C...-.-...G..A..G....C.A.AGA...T....-	30659
Query	595	GA-TGCTAACGATAAACAAGATGCTTTGAATAAGCAAATGCTGATCATAAAGATGAGTT	653
HE970764	22814	..-.....	22756
CP002618	22815	..-.....	22757
CP002616	22815	..-.....	22757

FM177140	22817	..-.....	22759
CP006690	21656	..-.....A.....	21598
CP001084	22165	..-.....A.....	22107
CP005486	20336	..-.....A.....	20278
CP005484	24697	..CC..A.G...G.....C..AG.....C.....C...C..A..	24638
AP012544	20972	..CA...G...C...G.....C.....CAA...C..CCGT..C..A..	21031
AP011548	30658	..CC..A.G...G.....C..AG.....C.....C...C..A..	30599
FM179322	30658	..CC..A.G...G.....C..AG.....C.....C...C..A..	30599
Query	654	GGTTGCTTTGCAAAGCCAATTTGCTCAAGAACAGTCTGAAG-CAGCTGCTGCAACCCAAG	712
HE970764	22755-.....	22697
CP002618	22756-.....	22698
CP002616	22756-.....	22698
FM177140	22758-.....	22700
CP006690	21597C.....C.....-.....	21539
CP001084	22106C.....C.....-.....	22048
CP005486	20277C.....C.....-.....	20219
CP005484	24637AC.C.....T.....A..A.....-.....CAAA.....G.	24579
AP012544	21032	A.....G...C..A..G.....-GAA...A.C.....G.....G.	21090
AP011548	30598AC.C.....T.....A..A.....-.....CAAA.....G.	30540
FM179322	30598AC.C.....T.....A..A.....-.....CAAA.....G.	30540
Query	713	CCGCTTTGAAGACCGTTGCCGCTTCAAC--C-GCAAGTTCTTCTA-C-----T-----A	757
HE970764	22696--,-.....-.....-.....-.....	22652
CP002618	22697--,-.....-.....-.....-.....	22653
CP002616	22697--,-.....-.....-.....-.....	22653
FM177140	22699--,-.....-.....-.....-.....	22655
CP006690	21538--,-.....-.....-.....-.....	21494
CP001084	22047--,-.....-.....-.....-.....	22003
CP005486	20218--,-.....-.....-.....-.....	20174
CP005484	24578	..T..C.....A.CG..T..A.....TG.TT.....A..G-----C-----	24529
AP012544	21091	..T.....G..C..T..A.....--,-.GTTC.G.A..A.G.AAGAG.GCCGAA.	21147
AP011548	30539	..T..C.....A.CA..T..A.....TG.TT.....A.C.G-----CA----	30489
FM179322	30539	..T..C.....A.CA..T..A.....TG.TT.....A.C.G-----CA----	30489
Query	758	C--C---AGCAGT-TC-A-AGC-AGCG-CAACTTCAA-CC--AGCAC-C-A-TCG---C	797
HE970764	22651-.....-.....-.....-.....-.....-.....-.....	22612
CP002618	22652-.....-.....-.....-.....-.....-.....-.....	22613
CP002616	22652-.....-.....-.....-.....-.....-.....-.....	22613
FM177140	22654-.....-.....-.....-.....-.....-.....-.....	22615
CP006690	21493-.....-.....-.....-.....-.....-.....-.....	21454
CP001084	22002	..-T-----	21963
CP005486	20173	..-T-----	20134
CP005484	24528	A--,----TT..AA-,-,-..-..-..T.C...AG..G-TG--TT.T.T.G.C.G.TAC.	24484
AP012544	21148	G--,----.....-..A.C.A.T.AG.-.TG.....G..AA.....-C..T---	21193
AP011548	30488	..TT.AAAT.AG...GC.A.C...-..T.-TTCT...G.-T-G.T...-...-.....	30444
FM179322	30488	..TT.AAAT.AG...GC.A.C...-..T.-TTCT...G.-T-G.T...-...-.....	30444
Query	798	-CAACAACACATCAAGTTCTTCAAACAACAGTTCTC--C-A-T---T-ATCAACAACAA	848
HE970764	22611-.....-.....-.....-.....-.....	22561
CP002618	22612-.....-.....-.....-.....-.....	22562
CP002616	22612-.....-.....-.....-.....-.....	22562
FM177140	22614-.....-.....-.....-.....-.....	22564
CP006690	21453T...-T-G-,-...-...C.....	21403
CP001084	21962T...-T-G-,-...-...C.....	21912
CP005486	20133T...-T-G-,-...-...C.....	20083
CP005484	24483	T..T...-..A.-.C...C..C...G.G..G...AT.T.C.G--.G...G..G...	24428
AP012544	21194	..-..-...G.-.CC...C...G.G..G...AT.-.-.CCG.C...G...G...C	21247
AP011548	30443	..T...-..A.-.C...C...G.G..G...AT.T.C.G--.G...G..G...	30389
FM179322	30443	..T...-..A.-.C...C...G.G..G...AT.T.C.G--.G...G..G...	30389
Query	849	CACGACTTCAGGTAGCGGCAGTACGCTGATTACAGCAGTTCAGGCAACACGTATCCTTG	908
HE970764	22560	22501
CP002618	22561	22502
CP002616	22561	22502
FM177140	22563	22504
CP006690	21402	..AG.....C..T...C.....C.T..TG...T...C.....	21343
CP001084	21911	..AG.....C..T...C.....C.T..TG...T...C.....	21852
CP005486	20082	..AG.....C..T...C.....C.T..TG...T...C.....	20023
CP005484	24427	..TG.....C..T...G.....T...T.....	24368
AP012544	21248	..CG.....C..T...T.C.....T.....	21307
AP011548	30388	..TG.....C..T...G.....T...T.....	30329
FM179322	30388	..TG.....C..T...G.....T...T.....	30329

		P40_casrham_for1	P40_casrham_for2	
Query	909	GGGTCAGTGCACCTGGTACGTCAAGTCAGTCGCTTCATGGGCTGGTAATGGCTGGGGCAA		968
HE970764	22500	22441
CP002618	22501	22442
CP002616	22501	22442
FM177140	22503	22444
CP006690	21342A.....T..AC.....	21283
CP001084	21851A.....T..AC.....	21792
CP005486	20022A.....T..AC.....	19963
CP005484	24367A.....T...T...T.....A..C.....	24308
AP012544	21308A.....T...T.....A..C.....	21367
AP011548	30328A.....T...T...T.....A..C.....	30269
FM179322	30328A.....T...T...T.....A..C.....	30269
Query	969	TGGTGCCCAATGGGGTAGTTCGGCTGCAGCTGCTGGTTTCACAGTCAACCACACGCCAGC		1028
HE970764	22440	22381
CP002618	22441	22382
CP002616	22441	22382
FM177140	22443	22384
CP006690	21282	C..C..A.....C....A..C....A....C....T..T.....	21223
CP001084	21791	C..C..A.....C....A..C....A....C....T..T.....	21732
CP005486	19962	C..C..A.....C....A..C....A....C....T..T.....	19903
CP005484	24307	C.....TG.....CGC.....A..C..C.....G..T.....C..G..	24248
AP012544	21368	C..C..TG.....CGC.....A..C..G..T.....A..G..	21427
AP011548	30268	C.....TG.....CGC.....A..C..C.....G..T.....C..G..	30209
FM179322	30268	C.....TG.....CGC.....A..C..C.....G..T.....C..G..	30209
Query	1029	AGCCGGTTCAATCATCGTCTTCGCCGCTGGTCAATCTGTTGGCGGTCAATGGACAGCCGA		1088
HE970764	22380	22321
CP002618	22381	22322
CP002616	22381	22322
FM177140	22383	22324
CP006690	21222C.....C.....	21163
CP001084	21731C.....C.....	21672
CP005486	19902C.....C.....	19843
CP005484	24247	..A.....C.....T.....T.....	24188
AP012544	21428C.....T.....T.....C....A.....C.....T..T..	21487
AP011548	30208	..A.....C.....T.....C.....T.....	30149
FM179322	30208	..A.....C.....T.....C.....T.....	30149
Query	1089	TGGTTCATACGGTACGTTGCTTACGTCCAATCTGTCTCTGGTGACAGTGCACGATCAG		1148
HE970764	22320	22261
CP002618	22321	22262
CP002616	22321	22262
FM177140	22323	22264
CP006690	21162T.....T.....T.....	21103
CP001084	21671T.....T.....T.....	21612
CP005486	19842T.....T.....T.....	19783
CP005484	24187	..C..T..T.....T..T...C..T...C....C..T.....C	24128
AP012544	21488	..CA..T.....T.....C..T..C..C....C...C..TTC	21547
AP011548	30148	..C..T..T.....T..T...C...C...C...T...C	30089
FM179322	30148	..C..T..T.....T..T...C...C...C...T...C	30089
Query	1149	CCAAGGCGGCATGGGCTTCAGCTCACCAACCGGCCCGAACACCCAAACCATCTCTGGTG		1208
HE970764	22260	22201
CP002618	22261	22202
CP002616	22261	22202
FM177140	22263	22204
CP006690	21102	21043
CP001084	21611	21552
CP005486	19782	19723
CP005484	24127	T.....T.....T.....G.....	24068
AP012544	21548G.....C..	21607
AP011548	30088	T.....T.....T.....G.....	30029
FM179322	30088	T.....T.....T.....G.....	30029
Query	1209	AAGCAGTTACGTTTATATCCACCGGTAA		1236
HE970764	22200	22173
CP002618	22201	22174
CP002616	22201	22174
FM177140	22203	22176
CP006690	21042C.....	21015
CP001084	21551C.....	21524

CP005486	19722C.....	19695
CP005484	24067	C.....C.....C.....	24040
AP012544	21608C...AC.....	21635
AP011548	30028	C.....C.....C.....	30001
FM179322	30028	C.....C.....C.....	30001

Table S5.- Blast alignment of *L.casei/paracasei/rhamnosus* genes encoding P75 proteins for the selection of PCR primers

Database: Nucleotide collection (nt)
 36,527,618 sequences; 117,866,738,766 total letters
 Query= gi|191636824|ref|NC_010999.1| Lactobacillus casei BL23 complete genome, strain BL23

Length=3079196

Sequences producing significant alignments:	Score (Bits)	E Value
emb HE970764.1 Lactobacillus casei W56 complete genome	2743	0.0
gb CP002618.1 Lactobacillus casei BD-II, complete genome	2743	0.0
gb CP002616.1 Lactobacillus casei LC2W, complete genome	2743	0.0
emb FM177140.1 Lactobacillus casei BL23 complete genome, str...	2743	0.0
gb CP006690.1 Lactobacillus casei 12A, complete genome	2604	0.0
gb CP001084.2 Lactobacillus casei str. Zhang, complete genome	2379	0.0
gb CP005486.1 Lactobacillus casei LOCK919, complete genome	2379	0.0
gb CP005484.1 Lactobacillus rhamnosus LOCK900, complete genome	420	2e-116
dbj AP011548.1 Lactobacillus rhamnosus ATCC 53103 DNA, compl...	420	2e-116
emb FM179322.1 Lactobacillus rhamnosus GG whole genome seque...	420	2e-116
gb CP014645.1 Lactobacillus rhamnosus strain ASCC 290 genome	409	4e-113
emb LT220504.1 Lactobacillus rhamnosus strain BPL5 genome as...	409	4e-113
gb CP005485.1 Lactobacillus rhamnosus LOCK908, complete genome	409	4e-113
gb CP003094.1 Lactobacillus rhamnosus ATCC 8530, complete ge...	409	4e-113
emb FM179323.1 Lactobacillus rhamnosus Lc 705 whole genome s...	409	4e-113

ALIGNMENTS

Query	277055	ATGGTAGATGCAAAGAAAGTATTGTCTACGTTACGGCAGGATTCGTTGGTGCTGCCGGTTTG	277114
HE970764	277026	277085
CP002618	274360	274419
CP002616	277061	277120
FM177140	277055	277114
CP006690	294753	294812
CP001084	294624	294683
CP005486	290686	290745
CP005484	295348T.T.....A.....C.....C..	295407
AP011548	324696T.T.....A.....C.....C..	324755
FM179322	325672T.T.....A.....C.....C..	325731
CP014645	102073T.T.....A.....C.....C..	102132
LT220504	1953631T.T.....A.....C.....C..	1953572
CP005485	315086T.T.....A.....C.....C..	315145
CP003094	307877T.T.....A.....C.....C..	307936
FM179323	307701T.T.....A.....C.....C..	307760
Query	277115	GCAACTTAGCTACCGCGCTAATACTGTTCCGCTTCAACGGGGACAGTCAATTACAAA	277174
HE970764	277086	277145
CP002618	274420	274479
CP002616	277121	277180
FM177140	277115	277174
CP006690	294813	294872
CP001084	294684	294743
CP005486	290746	290805
CP005484	295408	..GG.....A.....A..C.....C.....T..A..G..A.....G.....G.....	295467
AP011548	324756	..GG.....A.....A..C.....C.....T..A..G..A.....G.....G.....	324815
FM179322	325732	..GG.....A.....A..C.....C.....T..A..G..A.....G.....G.....	325791
CP014645	102133	..GG.....A.....A..C.....C.....T..A..G..A.....G.....G.....	102192
LT220504	1953571	..GG.....A.....A..C.....C.....T..A..G..A.....G.....G.....	1953512
CP005485	315146	..GG.....A.....A..C.....C.....T..A..G..A.....G.....G.....	315205
CP003094	307937	..GG.....A.....A..C.....C.....T..A..G..A.....G.....G.....	307996

FM179323	307761	..GG.....A.....A..C.....C.....T..A..G..A.....G.....G.....	307820
Query	277175	TCGGGTGC GACCACCGTATGGAATAGTCC ATCATGGCATCAAGTAAACGCTATGTGACT	277234
HE970764	277146	277205
CP002618	274480	274539
CP002616	277181	277240
FM177140	277175	277234
CP006690	294873	294932
CP001084	294744	294803
CP005486	290806	290865
CP005484	295468	..C.....C.....C.....C.....	295527
AP011548	324816	..C.....C.....C.....C.....	324875
FM179322	325792	..C.....C.....C.....C.....	325851
CP014645	102193	..C.....C.....C.....C.....	102252
LT220504	1953511	..C.....C.....C.....C.....	1953452
CP005485	315206	..C.....C.....C.....C.....	315265
CP003094	307997	..C.....C.....C.....C.....	308056
FM179323	307821	..C.....C.....C.....C.....	307880
Query	277235	TTTGGTGACAAAGTACAAGTTCTAGGTCAAAAAGTTGACCGAAACGGTGCTACTTGGTAT	277294
HE970764	277206	277265
CP002618	274540	274599
CP002616	277241	277300
FM177140	277235	277294
CP006690	294933	294992
CP001084	294804	294863
CP005486	290866	290925
CP005484	295528C....CG..G..GC.AT....A...CC.....A..T.....	295587
AP011548	324876G....CG..G..GC.AT.G...A...CC.....A..T.....	324935
FM179322	325852G....CG..G..GC.AT.G...A...CC.....A..T.....	325911
CP014645	102253C....CG..G..GC.AT....A...CC.....A..T.....	102312
LT220504	1953451C....CG..G..GC.AT....A...CC.....A..T.....	1953392
CP005485	315266C....CG..G..GC.AT....A...CC.....A..T.....	315325
CP003094	308057C....CG..G..GC.AT....A...CC.....A..T.....	308116
FM179323	307881C....CG..G..GC.AT....A...CC.....A..T.....	307940
Query	277295	AAAGTC CGCGACAATCAGTGGATCC CTCATT-TATTGAATTTTGACGGCAAGACTGT	277353
HE970764	277266	277324
CP002618	274600	274658
CP002616	277301	277359
FM177140	277295	277353
CP006690	294993GG.....	295051
CP001084	294864GG.....	294922
CP005486	290926GG.....	290984
CP005484	295588T.....T...GGA-...G.....G...CG..T..A..G.C	295646
AP011548	324936T.....T...GGA-...G.....G...CG..T..A..G.C	324994
FM179322	325912T.....T...GGA-...G.....G...CG..T..A..G.C	325970
CP014645	102313T.....T...GGA-...G.....G...CG..T..A..G.C	102371
LT220504	1953391T.....T...GGA-...G.....G...CG..T..A..G.C	1953333
CP005485	315326T.....T...GGA-...G.....G...CG..T..A..G.C	315384
CP003094	308117T.....T...GGA-...G.....G...CG..T..A..G.C	308175
FM179323	307941T.....T...GGA-...G.....G...CG..T..A..G.C	307999
Query	277354	CACCGTTC CAAGCACC-AGAACA AACTGCCAGTCAGGCACCGGTTAGCCAAGCGCCAGCTA	277412
HE970764	277325	277383
CP002618	274659	274717
CP002616	277360	277418
FM177140	277354	277412
CP006690	295052	295110
CP001084	294923	294981
CP005486	290985	291043
CP005484	295647G...G..A...G..TT.GG-.A..A...AA.TG.T..C.....A..G...	295705
AP011548	324995G...G..A...G..TT.GG-.A..A...AA.TG.T..C.....A..G...	325053
FM179322	325971G...G..A...G..TT.GG-.A..A...AA.TG.T..C.....A..G...	326029
CP014645	102372G...G..A...G..TT.GG-.A..A...AA.TG.T..C.....A..G...	102430
LT220504	1953332G...G..A...G..TT.GG-.A..A...AA.TG.T..C.....A..G...	1953274
CP005485	315385G...G..A...G..TT.GG-.A..A...AA.TG.T..C.....A..G...	315443
CP003094	308176G...G..A...G..TT.GG-.A..A...AA.TG.T..C.....A..G...	308234
FM179323	308000G...G..A...G..TT.GG-.A..A...AA.TG.T..C.....A..G...	308058
Query	277413	GCCAAGCACCTG CAAGTCAGGCACCTGCGAGTCAAGCAGC--C-GCAC-AG--CCTGATA	277466
HE970764	277384	277437
CP002618	274718	274771

CP002616	277419--.-.....	277472
FM177140	277413--.-.....	277466
CP006690	295111--.-.....	295164
CP001084	294982--.-.....	295035
CP005486	291044--.-.....	291097
CP005484	295706	.T.G.G.A...C.A...A.A.CC..A.C.TG.A...C.CAAA.C...	295765
AP011548	325054	.T.G.G.A...C.A...A.A.CC..A.C.TG.A...C.CAAA.C...	325113
FM179322	326030	.T.G.G.A...C.A...A.A.CC..A.C.TG.A...C.CAAA.C...	326089
CP014645	102431	.T.G.G.A...C.A...A.A.CC..A.C.TG.A...C.CAAA.C...	102490
LT220504	1953273	.T.G.G.A...C.A...A.A.CC..A.C.TG.A...C.CAAA.C...	1953214
CP005485	315444	.T.G.G.A...C.A...A.A.CC..A.C.TG.A...C.CAAA.C...	315503
CP003094	308235	.T.G.G.A...C.A...A.A.CC..A.C.TG.A...C.CAAA.C...	308294
FM179323	308059	.T.G.G.A...C.A...A.A.CC..A.C.TG.A...C.CAAA.C...	308118
Query	277467	CCCAGACAGC-AA-ACATTCAGTTATATGTTAAAAACATTGGTTCTGCAGTCACAGTTG	277524
HE970764	277438-.-.....	277495
CP002618	274772-.-.....	274829
CP002616	277473-.-.....	277530
FM177140	277467-.-.....	277524
CP006690	295165-.-.....	295222
CP001084	295036-C.....C.....	295093
CP005486	291098-C.....C.....	291155
CP005484	295766	...A...T.T...--..C.T...C.G.T.C...A...T.C.A..	295823
AP011548	325114	...A...T.T...--..C.T...C.G.T.C...A...T.C.A..	325171
FM179322	326090	...A...T.T...--..C.T...C.G.T.C...A...T.C.A..	326147
CP014645	102491	...A...T.T...--..C.T...C.G.T.C...A...T.C.A..	102548
LT220504	1953213	...A...T.T...--..C.T...C.G.T.C...A...T.C.A..	1953156
CP005485	315504	...A...T.T...--..C.T...C.G.T.C...A...T.C.A..	315561
CP003094	308295	...A...T.T...--..C.T...C.G.T.C...A...T.C.A..	308352
FM179323	308119	...A...T.T...--..C.T...C.G.T.C...A...T.C.A..	308176
Query	277525	GGCAACACCAGCGTACAGTCAGGCAACTGGCCAGTATCTGGAAGGCAACCAGACGTTGAC	277584
HE970764	277496	277555
CP002618	274830	274889
CP002616	277531	277590
FM177140	277525	277584
CP006690	295223	295282
CP001084	295094	295153
CP005486	291156	291215
CP005484	295824	.A...C.G...T.C...T.G.A.T.A...A...G...AC...	295883
AP011548	325172	.A...C.G...T.C...T.G.A.T.A...A...G...AC...	325231
FM179322	326148	.A...C.G...T.C...T.G.A.T.A...A...G...AC...	326207
CP014645	102549	.A...C.G...T.C...T.G.A.T.A...A...G...AC...	102608
LT220504	1953155	.A...C.G...T.C...T.G.A.T.A...A...G...AC...	1953096
CP005485	315562	.A...C.G...T.C...T.G.A.T.A...A...G...AC...	315621
CP003094	308353	.A...C.G...T.C...T.G.A.T.A...A...G...AC...	308412
FM179323	308177	.A...C.G...T.C...T.G.A.T.A...A...G...AC...	308236
Query	277585	GGCGGTTGCCAGTTGCAAGCCAATGGCGAAACATGGTATCGGTTAGCAAATGGCGGTTA	277644
HE970764	277556	277615
CP002618	274890	274949
CP002616	277591	277650
FM177140	277585	277644
CP006690	295283	295342
CP001084	295154	295213
CP005486	291216	291275
CP005484	295884	..T.....CA...A.C...C.T.C.C.....	295943
AP011548	325232	..T.....CA...A...T.....C.T.C.C.....	325291
FM179322	326208	..T.....CA...A...T.....C.T.C.C.....	326267
CP014645	102609	..T.....CA...A...T.....G.....C.T.C.C.....	102668
LT220504	1953095	..T.....CA...A...T.....G.....C.T.C.C.....	1953036
CP005485	315622	..T.....CA...A...T.....G.....C.T.C.C.....	315681
CP003094	308413	..T.....CA...A...T.....G.....C.T.C.C.....	308472
FM179323	308237	..T.....CA...A...T.....G.....C.T.C.C.....	308296
Query	277645	CGTGCCAGAACGTTTTGCTAGCACAAACCTGCACCAGCACCAGTCATCTGCTGCAAC	277704
HE970764	277616	277675
CP002618	274950	275009
CP002616	277651	277710
FM177140	277645	277704
CP006690	295343	295402
CP001084	295214	295273
CP005486	291276	291335

CP005484	295944	T..T..T.C...G....T.....	295973
AP011548	325292	T..T..T.C...G....T.....	325321
FM179322	326268	T..T..T.C...G....T.....	326297
CP014645	102669	T..T..T.C...G....T.....	102695
LT220504	1953035	T..T..T.C...G....T.....G..	1953006
CP005485	315682	T..T..T.C...G....T.....	315708
CP003094	308473	T..T..T.C...G....T.....	308499
FM179323	308297	T..T..T.C...G....T.....	308323
Query	277705	GAGTGTTCGGCACCCTGCGCCTGTATCTGATGCAACGGCTTCGAACGCAGCTGCTTC	277764
HE970764	277676	277735
CP002618	275010	275069
CP002616	277711	277770
FM177140	277705	277764
CP006690	295403	295462
CP001084	295274	295328
CP005486	291336	291390
Query	277765	AAATGCCGCCGCTTCGGATGTTGCTGTCTCCAGTGCTGCAGCGTCTAGTGCCCGGGCTTC	277824
HE970764	277736	277795
CP002618	275070	275129
CP002616	277771	277830
FM177140	277765	277824
CP006690	295463	295522
CP001084	295329	-----C.....C.....C.....C.....T..A.....	295378
CP005486	291391	-----C.....C.....C.....C.....T..A.....	291440
Query	277825	ATTAGTGCCGCTTCAGAAGCTGCTG---CGGTTGCGAATGCTTCTAGCGCTGCTGCATC	277881
HE970764	277796	277852
CP002618	275130	275186
CP002616	277831	277887
FM177140	277825	277881
CP006690	295523	295579
CP001084	295379	...G...T.....CTT.....A.....C.....	295438
CP005486	291441	...G...T.....CTT.....A.....C.....	291500
Query	277882	-T-T-CTGC--T-GC-----C-GCTGTAG---CGAGTGCTGCCGCAGAAATCCAGTGCTGC	277926
HE970764	277853	-.-.------.------.-----	277897
CP002618	275187	-.-.------.------.-----	275231
CP002616	277888	-.-.------.------.-----	277932
FM177140	277882	-.-.------.------.-----	277926
CP006690	295580	-.-.------.------.-----	295606
CP001084	295439	G.C.G...TG.A..GAGTG.T..C.C..AAT.C...A.G..T.....	295498
CP005486	291501	G.C.G...TG.A..GAGTG.T..C.C..AAT.C...A.G..T.....	291560
Query	277927	TGCAGAGTCCAGTGCTGCCGCATCGAAGGCTGCTGCTGATTCTAGCGCTGCAGCTGTGCA	277986
HE970764	277898	277957
CP002618	275232	275291
CP002616	277933	277992
FM177140	277927	277986
CP006690	295607	295666
CP001084	295499A.....T.....T.....	295558
CP005486	291561A.....T.....T.....	291620
Query	277987	GACTACGACGCCTGAGTCCAGTGCTGCGCCTGCAACGACACAGGTTGATGCAACTCAGGA	278046
HE970764	277958	278017
CP002618	275292	275351
CP002616	277993	278052
FM177140	277987	278046
CP006690	295667	295726
CP001084	295559	295618
CP005486	291621	291680
Query	278047	ACAGCAACAGCAGGCAGAACCAAGCAATACGGTCAATACCGAGGAAACGACAAATAACGC	278106
HE970764	278018	278077
CP002618	275352	275411
CP002616	278053	278112
FM177140	278047	278106
CP006690	295727	295786
CP001084	295619T.....G.....	295678
CP005486	291681T.....G.....	291740
Query	278107	GACGCCGACTCCTGCACCAACGCCGACGCTGCACCAACCCAGCCAGCTCCTGTAC	278166

HE970764	278078	278137
CP002618	275412	275471
CP002616	278113	278172
FM177140	278107	278166
CP006690	295787	295846
CP001084	295679-----	295732
CP005486	291741-----	291794
Query	278167	GCCGTCACGTCAGGCCAAAAATTCAGGCGGTTATCTCAATTGCTGAACAGCAAATTGGGAA	278226
HE970764	278138	278197
CP002618	275472	275531
CP002616	278173	278232
FM177140	278167	278226
CP006690	295847G.....	295906
CP001084	295733	295792
CP005486	291795	291854
Query	278227	ACCTTATGTATGGGGTGGCAAGGGTCTTAACAGCTTTGACTGCTCAGGCTTGATGTATTA	278286
HE970764	278198	278257
CP002618	275532	275591
CP002616	278233	278292
FM177140	278227	278286
CP006690	295907	295966
CP001084	295793	295852
CP005486	291855	291914
Query	278287	TGCCTTTTTGAACGGCGCCGGTGTAAACATCGGTGGTTGGACAGTGCCACAAGAATCTTC	278346
HE970764	278258	278317
CP002618	275592	275651
CP002616	278293	278352
FM177140	278287	278346
CP006690	295967	296026
CP001084	295853	295912
CP005486	291915	291974
Query	278347	TGGTCAGCAAGTCTCACTGAGTGCAC TTCAGCCTGGTGATTGCTCTTCTGGGGCGGACA	278406
HE970764	278318	278377
CP002618	275652	275711
CP002616	278353	278412
FM177140	278347	278406
CP006690	296027	296086
CP001084	295913	295972
CP005486	291975	292034
Query	278407	TGGTAGCTCTTACCACGTAGCGCTCTACATTGGTGGCGGTACAATGATT CAGGCACCACA	278466
HE970764	278378	278437
CP002618	275712	275771
CP002616	278413	278472
FM177140	278407	278466
CP006690	296087	296146
CP001084	295973	296032
CP005486	292035	292094
Query	278467	ACCAGGTGAAAATGTGAAGTACACGGCATTAGCTTACTTCATGCCTGATTTTGCTGTTCG	278526
HE970764	278438	278497
CP002618	275772	275831
CP002616	278473	278532
FM177140	278467	278526
CP006690	296147T.....	296206
CP001084	296033T.....	296092
CP005486	292095T.....	292154
Query	278527	TCCTTCACTATAA	278539
HE970764	278498	278510
CP002618	275832	275844
CP002616	278533	278545
FM177140	278527	278539
CP006690	296207	296219
CP001084	296093	296105
CP005486	292155	292167



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