Supplementary material

Simultaneous presence of PrtH and PrtH2 proteinases in *Lactobacillus helveticus* strains improves breakdown of the pure α_{s1}-casein

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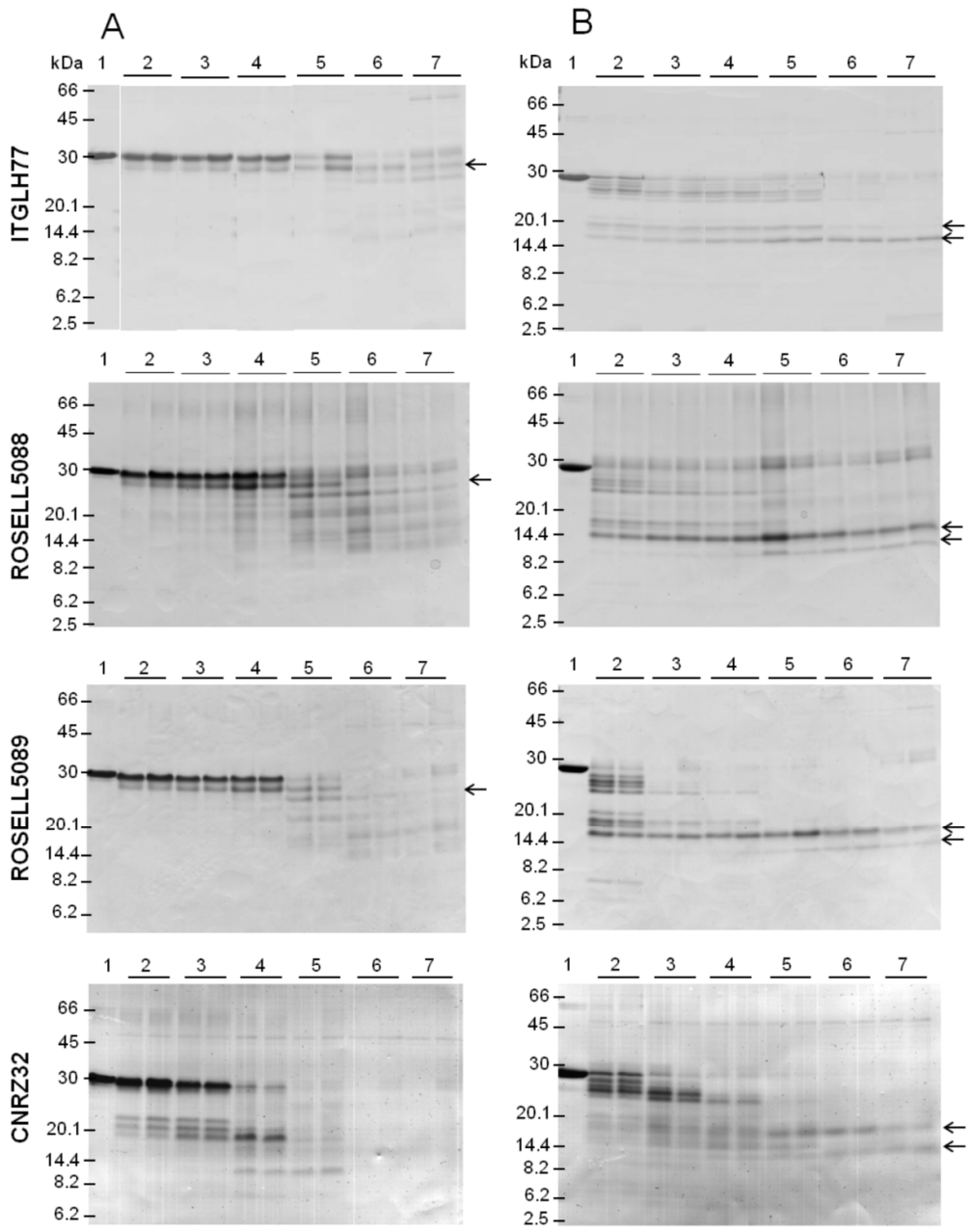

FIG. S1. Hydrolysis of purified α_{s1}-casein (A) and β-casein (B) by strains of *L. helveticus* after growth in Low Low Heated milk. Caseins were incubated at 40°C with washed-cell suspensions of *L. helveticus* strains. Samples were taken after 0 min (lane 1), 5 min (lane 2), 15 min (lane 3), 30 min (lane 4), 60 min (lane 5), 120 min (lane 6) and 180 min (lane7) of hydrolysis. Cells were removed by centrifugation, and the supernatants were analysed by SDS-PAGE. Experiments were performed in duplicate. Names of the strains are indicated on the left of the gels. The arrow indicated peptides which were present all the incubation time long.

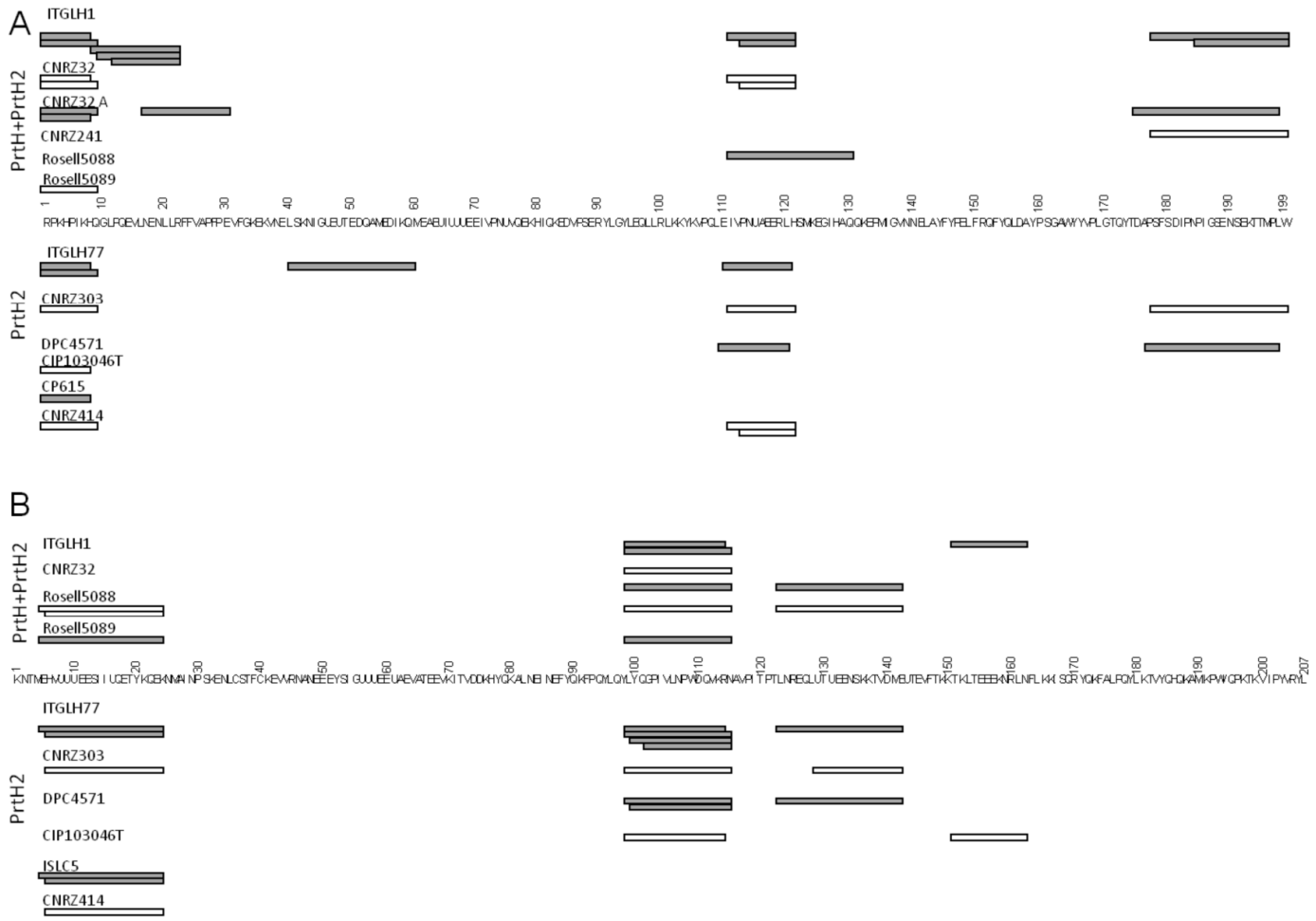

FIG. S2. Degradation profiles of (A) α_{s1}-casein, (B) α_{s2}-casein and (C) β-casein by *L. helveticus* strains grown in UHT milk. Rectangles correspond to peptides identified by mass spectrometry after centrifugation and filtration of acidified milk. U designates a phosphoserine residue.

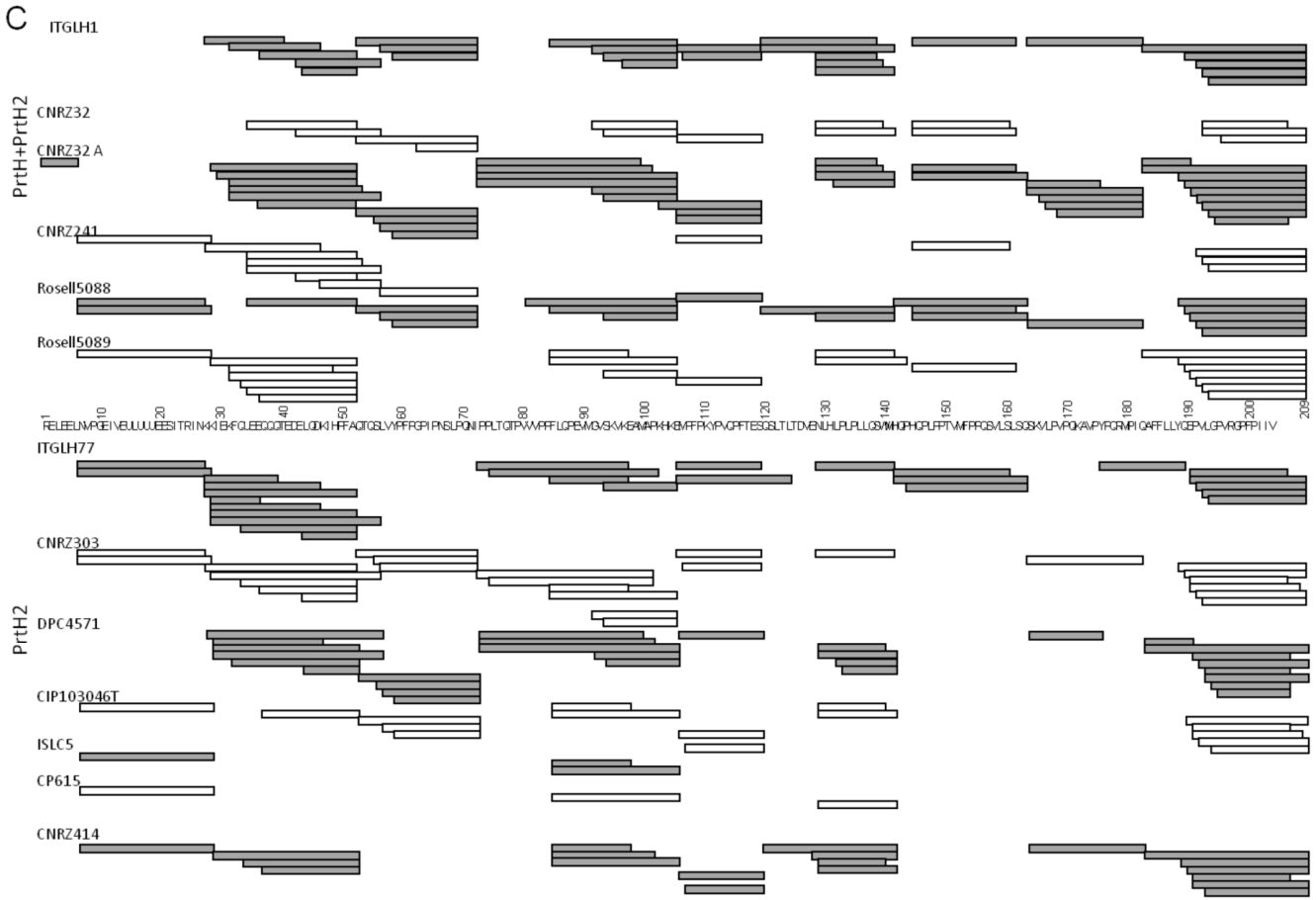

FIG. S2 (continued)

TABLE S1. Peptides identified from α_{s1}-casein hydrolysis by four strains of *L. helveticus;* peptides released from purified casein (P) or from milk (M). Strains having two CEPs (ITGLH1 and Rosell5088) and strains having only one CEP (ITGLH77 and ISLC5) are shown.

α_{s1}-CN Peptides	ITG LH1		Rosell 5088		ITG LH77		ISLC5	
	P	M	P	M	P	M	P	M
α_{s1}-CN (1-7)			■					
α_{s1}-CN (1-8)		■				■		
α_{s1}-CN (1-9)		■				■		
α_{s1}-CN (8-14)	■							
α_{s1}-CN (8-15)					■			
α_{s1}-CN(8-16)							■	
α_{s1}-CN (8-19)							■	
α_{s1}-CN (8-21)					■			
α_{s1}-CN (9-14)	■				■			
α_{s1}-CN (9-15)					■			
α_{s1}-CN (9-16)							■	
α_{s1}-CN (9-19)	■						■	
α_{s1}-CN (9-21)			■					
α_{s1}-CN (9-22)		■	■		■			
α_{s1}-CN (10-22)		■	■					
α_{s1}-CN (12-22)		■						
α_{s1}-CN (13-22)			■					
α_{s1}-CN (14-22)	■				■		■	
α_{s1}-CN (14-23)	■						■	
α_{s1}-CN (17-22)			■					
α_{s1}-CN (17-23)							■	
α_{s1}-CN (24-30)	■				■		■	
α_{s1}-CN (25-30)	■				■		■	
α_{s1}-CN (31-40)			■					
α_{s1}-CN (32-40)			■					
α_{s1}-CN (33-40)	■		■					
α_{s1}-CN (41-60) **2P**						■		
α_{s1}-CN (83-92)			■					
α_{s1}-CN (83-98)			■					
α_{s1}-CN (84-92)	■							
α_{s1}-CN (84-93)	■							
α_{s1}-CN (103-121) **1P**							■	
α_{s1}-CN (104-110)			■					
α_{s1}-CN (104-121) **1P**							■	
α_{s1}-CN (105-114)			■					
α_{s1}-CN (105-121) **1P**			■				■	
α_{s1}-CN (108-114)	■				■			
α_{s1}-CN (108-135) **1P**	■							
α_{s1}-CN (109-121) **1P**	■		■		■		■	
α_{s1}-CN (109-123) **1P**			■		■			
α_{s1}-CN (109-130) **1P**			■		■		■	
α_{s1}-CN (110-121) **1P**					■			
α_{s1}-CN (111-121) **1P**	■	■	■		■	■		
α_{s1}-CN (111-123) **1P**			■					
α_{s1}-CN (111-124) **1P**			■					
α_{s1}-CN (111-130) **1P**				■	■			
α_{s1}-CN (112-121) **1P**	■		■		■			
α_{s1}-CN (113-121) **1P**		■	■					

α_{s1}-CN (115-123) **1P**	■		■					
α_{s1}-CN (115-124) **1P**			■					
α_{s1}-CN (115-130) **1P**			■					
α_{s1}-CN (122-130)			■					
α_{s1}-CN (131-138)			■					
α_{s1}-CN (131-139)	■		■					
α_{s1}-CN (131-142)			■		■		■	
α_{s1}-CN (131-143)			■					
α_{s1}-CN (132-142)			■					
α_{s1}-CN (136-142)	■						■	
α_{s1}-CN (139-148)			■					
α_{s1}-CN (170-189)	■							
α_{s1}-CN (173-189)	■							
α_{s1}-CN (178-199)		■						
α_{s1}-CN (179-192)	■							
α_{s1}-CN (179-199)			■					
α_{s1}-CN (180-189)	■							
α_{s1}-CN (185-199)		■						
α_{s1}-CN (187-199)			■					
Total number of	22	9	34	1	1	4	16	0

■, peptide present; 1P or 2P correspond to the presence of 1 or 2 phosphorylated residues in the peptide.

TABLE S2 Peptides identified from β-casein hydrolysis by four strains of *L. helveticus*; peptides released from purified casein (P) or in milk (M). Strains having two CEPs (ITGLH1 and Rosell5088) and strains having only one CEP (ITGLH77 and ISLC5) are shown.

β-CN Peptides	ITG LH1		Rosell 5088		ITG LH77		ISLC5	
	P	M	P	M	P	M	P	M
β-CN (1-6)					■			
β-CN (2-6)	■							
β-CN (7-27) **4P**				■		■		
β-CN (7-28) **4P**				■		■		■
β-CN (28-39) **1P**						■		
β-CN (28-40) **1P**		■						
β-CN (28-46) **1P**	■					■		
β-CN (28-52) **1P**					■	■		
β-CN (29-36) **1P**						■		
β-CN (29-46) **1P**						■		
β-CN (29-52) **1P**						■		
β-CN (29-56) **1P**						■		
β-CN (31-52) **1P**			■					
β-CN (32-46) **1P**		■						
β-CN (34-52) **1P**	■					■		
β-CN (35-52)				■				
β-CN (37-52)		■						
β-CN (43-56)		■						
β-CN (44-52)		■				■		
β-CN (47-52)	■							
β-CN (53-72)		■		■				
β-CN (57-68)			■				■	
β-CN (57-72)		■	■	■				
β-CN (58-68)							■	
β-CN (59-68)							■	
β-CN (59-72)		■		■				
β-CN (66-72)	■							
β-CN (70-77)							■	
β-CN (73-79)	■						■	
β-CN (73-97)						■		
β-CN (73-105)			■		■		■	
β-CN (74-79)							■	
β-CN (74-82)	■						■	
β-CN (75-102)						■		
β-CN (78-91)							■	
β-CN (79-91)							■	
β-CN (80-91)							■	
β-CN (80-93)	■						■	
β-CN (80-96)	■							
β-CN (80-101)							■	
β-CN (81-105)				■				
β-CN (82-91)							■	
β-CN (83-91)							■	
β-CN (84-88)	■						■	
β-CN (84-91)	■							
β-CN (85-91)							■	
β-CN (85-97)						■		■
β-CN (85-105)		■		■				■

β-CN Peptides	ITG LH1		Rosell 5088		ITG LH77		ISLC5	
	P	M	P	M	P	M	P	M
β-CN (88-93)								
β-CN (92-105)								
β-CN (94-105)								
β-CN (97-105)								
β-CN (106-111)								
β-CN (106-119)								
β-CN (106-124)								
β-CN (106-134)								
β-CN (107-119)								
β-CN (108-119)								
β-CN (109-119)								
β-CN (111-119)								
β-CN (114-119)								
β-CN (120-127)								
β-CN (120-128)								
β-CN (120-138)								
β-CN (120-141)								
β-CN (124-131)								
β-CN (126-131)								
β-CN (126-133)								
β-CN (126-155)								
β-CN (127-141)								
β-CN (128-139)								
β-CN (128-141)								
β-CN (128-143)								
β-CN (129-136)								
β-CN (129-138)								
β-CN (129-139)								
β-CN (129-141)								
β-CN (129-142)								
β-CN (129-143)								
β-CN (129-146)								
β-CN (129-158)								
β-CN (130-139)								
β-CN (132-139)								
β-CN (132-141)								
β-CN (133-139)								
β-CN (134-139)								
β-CN (134-141)								
β-CN (135-139)								
β-CN (135-141)								
β-CN (141-148)								
β-CN (142-160)								
β-CN (142-163)								
β-CN (144-154)								
β-CN (144-163)								
β-CN (145-154)								
β-CN (145-161)								

β-CN Peptides	ITG LH1		Rosell 5088		ITG LH77		ISLC5	
	P	M	P	M	P	M	P	M
β-CN (145-163)				■				
β-CN (146-154)							■	
β-CN (148-154)	■				■		■	
β-CN (149-154)	■		■		■			
β-CN (155-161)			■		■			
β-CN (157-161)							■	
β-CN (161-167)							■	
β-CN (164-175)							■	
β-CN (164-182)		■		■				
β-CN (166-175)					■		■	
β-CN (167-175)					■		■	
β-CN (167-191)					■			
β-CN (168-175)					■			
β-CN (169-175)			■		■			
β-CN (170-175)			■		■			
β-CN (171-175)			■		■			
β-CN (176-189)						■		
β-CN (177-182)			■		■		■	
β-CN (183-189)					■			
β-CN (183-190)			■				■	
β-CN (183-209)		■						
β-CN (184-188)							■	
β-CN (184-190)					■		■	
β-CN (189-209)				■				
β-CN (190-209)		■		■				
β-CN (191-206)						■	■	
β-CN (191-209)				■		■		
β-CN (192-206)			■				■	
β-CN (192-209)		■		■		■		
β-CN (193-198)	■		■		■		■	
β-CN (193-201)	■		■		■		■	
β-CN (193-202)					■		■	
β-CN (193-206)			■		■		■	
β-CN (193-207)					■		■	
β-CN (193-209)		■	■	■		■		
β-CN (194-200)	■							
β-CN (194-201)	■		■				■	
β-CN (194-202)	■							
β-CN (194-206)	■		■		■		■	
β-CN (194-207)					■			
β-CN (194-209)		■	■		■	■	■	
β-CN (195-201)	■							
β-CN (195-204)	■							
β-CN (195-206)	■				■			
β-CN (203-209)							■	
Total number of peptides	32	27	36	21	42	27	58	3

■, peptide present; 1P or 4P correspond to the presence of 1 or 4 phosphorylated residues in the peptide.