

# MSc Thesis - Chapter 6:

# Figures and Tables

Ursula Louise Thomashoff

**Table 6.1** Descriptive labels of the yoghurt preparations and their respective symbols.

Culture added to yoghurt mix			Culture added to yoghurt mix		
YBU	–	Unadapted <i>B. bifidum</i>	YBA	–	Stress-adapted <i>B. bifidum</i>
YRU	–	Unadapted <i>B. breve</i>	YRA	–	Stress-adapted <i>B. breve</i>
YAU	–	Unadapted <i>B. animalis</i>	YAA	–	Stress-adapted <i>B. animalis</i>

**Table 6.2** Selective media composition for the enumeration of bacterial species from probiotic yoghurt.

Bacterial species	Media	Reference
<i>S. thermophilus</i>	M17 agar supplemented with 1% lactose (v/w)	Shah (2000)
<i>L. bulgaricus</i>	MRS agar adjusted to pH 5.4 with 0.13% (v/v) acetic acid	
<i>Bifidobacterium spp.</i>	MRS agar supplemented with nalidixic acid sodium salt (0.015 g/l), neomycin sulfate (0.001 g/l), lithium chloride (3 g/l), paromomycin sulfate (0.2 g/l), L-cysteine (5 g/l) (MRS-NNLP Agar)	

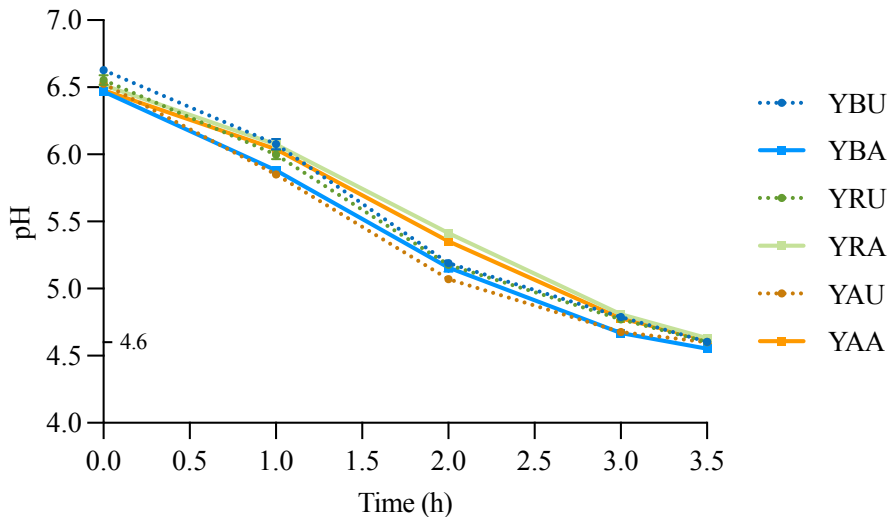
**Table 6.3** Species-specific primers for q-PCR of probiotics and yoghurt cultures.

Organism	Primer sequence		References
<i>S. thermophilus</i>	F	5'-CGTGGTGTTGTTTCGTGTTAATGA-3'	Fan et al. (2021)
	R	5'-CGGCAATACCTTCATCAAGTTGT-3'	
<i>L. delbrueckii</i>	F	5'-AGACTCTTGACTTGGGGTGAAGC-3'	Marole et al. (2024)
	R	5'-GTTCTGTGGGTCTTGATTGAGC-3'	
<i>Bifidobacterium</i> species	F	5'-AAGCCGTTTCCTGATGCCTATC-3'	Marole et al. (2024)
	R	5'-GAGGTAACGGTGGTGGTCTG-3'	

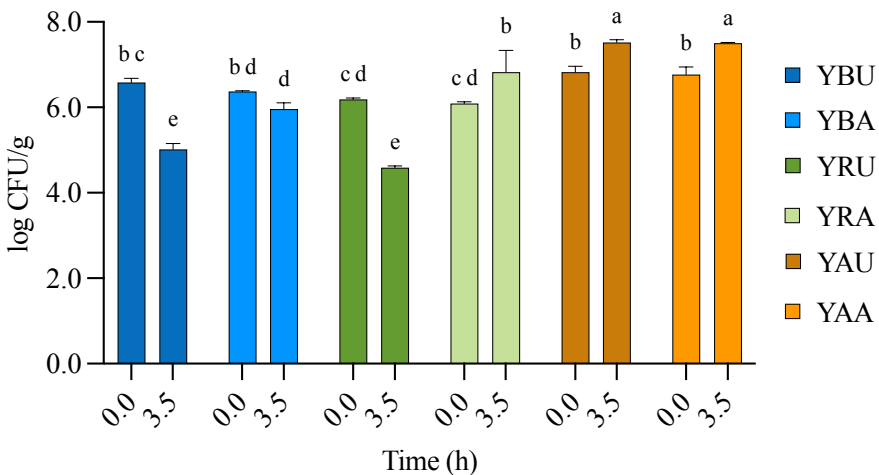
F: Forward Primer; R: Reverse Primer

**Table 6.4** Analysis of variance showing the effect of bacterial species and adaptation treatment on the pH and viability of unadapted or adapted *Bifidobacterium* spp. viability of probiotic yoghurt during fermentation (0 - 3.5 h).

Source of Variation	DF	P-Value	
		pH	Probiotic Viability
Main Effects			
<i>Bifidobacterium</i> spp. ( <i>B. bifidum</i> , <i>B. breve</i> , <i>B. animalis</i> )	2	< 0.001	< 0.001
Stress Adaptation (Unadapted, Adapted)	1	-	< 0.001
Fermentation Time (Probiotic Viability) (0, 3.5 h)	1	-	< 0.001
Interactions			
Spp. x Stress Adaptation	2	-	< 0.001
Spp. x Fermentation Time (Probiotic Viability)	2	-	< 0.001
Stress Adaptation x Fermentation Time (Probiotic Viability)	1	-	< 0.001
Spp. x Stress Adaptation x Fermentation Time (Probiotic Viability)	2	-	< 0.001



**Figure 6.1** Effect of stress adaptation on the pH (left) and *Bifidobacterium* spp. viability (right) during fermentation of yoghurt containing (A) *B. bifidum*, (B) *B. breve*, (C) *B. animalis*. Values are the means  $\pm$  standard deviation ( $n = 4$ ). YBU: yoghurt with unadapted *B. bifidum*; YBA: yoghurt with stress-adapted *B. bifidum*; YRU: yoghurt with unadapted *B. breve*; YRA: yoghurt with stress-adapted *B. breve*; YAU: yoghurt with unadapted *B. animalis*; YAA: yoghurt with stress-adapted *B. animalis*.

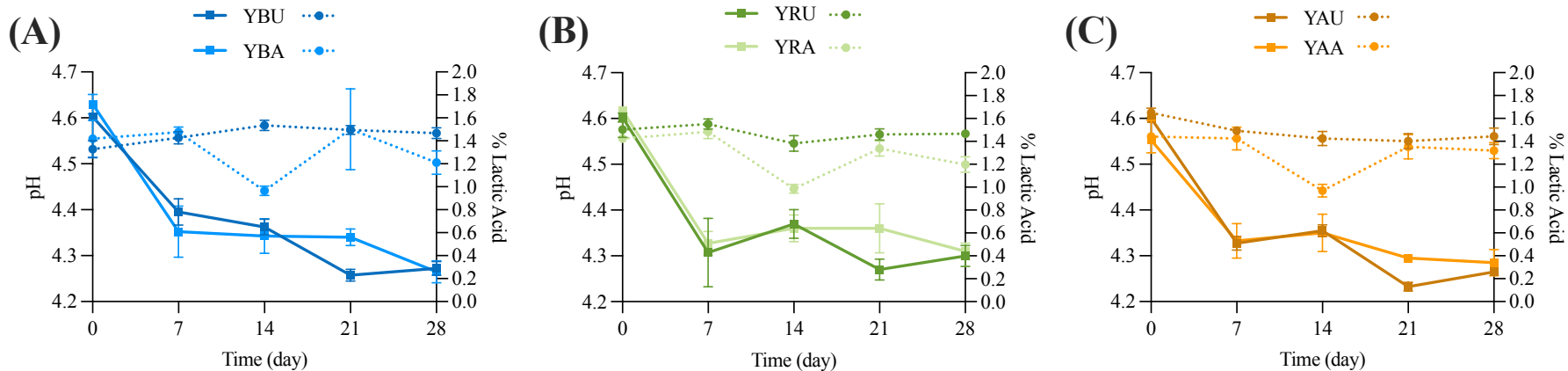


**Figure 6.2** Effect of stress adaptation on the *Bifidobacterium* spp. viability during fermentation of yoghurt containing *B. bifidum*, *B. breve* and *B. animalis*. Values are the means  $\pm$  standard deviation ( $n = 4$ ). YBU: yoghurt with unadapted *B. bifidum*; YBA: yoghurt with stress-adapted *B. bifidum*; YRU: yoghurt with unadapted *B. breve*; YRA: yoghurt with stress-adapted *B. breve*; YAU: yoghurt with unadapted *B. animalis*; YAA: yoghurt with stress-adapted *B. animalis*.

**Table 6.5** Analysis of variance showing the effect of bacterial species and adaptation treatment on the physicochemical characteristics (pH, TA,  $\Delta$ ORP, Syneresis) of yoghurt containing either unadapted or adapted *Bifidobacterium* spp., during 28 days of refrigerated storage.

Source of Variation	DF	P-Value		
		pH	TA	ΔORP
Main Effects				
<i>Bifidobacterium</i> spp. ( <i>B. bifidum</i> , <i>B. breve</i> , <i>B. animalis</i> )	2	< 0.001	-	< 0.001
Stress Adaptation (Unadapted, Adapted)	1	0.011	< 0.001	< 0.001
Shelf-life (pH, TA, Syneresis) (0, 7, 14, 21, 28 days)	4	< 0.001	< 0.001	-
Shelf-life (ΔORP) (7, 14, 21, 28 days)	3	-	-	< 0.001





**Figure 6.3** Effect of stress adaptation on the pH (left) and titratable acidity (% lactic acid) (right) over the 28-day cold storage (4 °C) period of yoghurt containing (A) *B. bifidum*, (B) *B. breve*, (C) *B. animalis*. Values are the means  $\pm$  standard deviation ( $n = 4$ ). YBU: yoghurt with unadapted *B. bifidum*; YBA: yoghurt with stress-adapted *B. bifidum*; YRU: yoghurt with unadapted *B. breve*; YRA: yoghurt with stress-adapted *B. breve*; YAU: yoghurt with unadapted *B. animalis*; YAA: yoghurt with stress-adapted *B. animalis*.

**Table 6.6** Change in ORP ( $\Delta mV$ ) over the 28-day cold storage (4 °C) period of yoghurt containing unadapted or adapted *Bifidobacterium* species.

Time (day)	Change in ORP ( $\Delta mV$ )					
	YBU	YBA	YRU	YRA	YAU	YAA
0 - 7	128.4 $\pm$ 23.9	140.8 $\pm$ 21.4	154.1 $\pm$ 5.7	79.9 $\pm$ 5.6	114.7 $\pm$ 2.8	49.9 $\pm$ 9.9
0 - 14	147.0 $\pm$ 25.6	160.7 $\pm$ 3.8	169.1 $\pm$ 6.9	110.4 $\pm$ 4.8	130.3 $\pm$ 1.3	80.3 $\pm$ 4.7
0 - 21	169.9 $\pm$ 25.5	171.8 $\pm$ 19.7	194.0 $\pm$ 6.9	120.6 $\pm$ 2.1	154.8 $\pm$ 1.2	97.5 $\pm$ 6.1
0 - 28	152.8 $\pm$ 26.1	168.8 $\pm$ 17.7	180.1 $\pm$ 7.1	120.2 $\pm$ 2.2	146.8 $\pm$ 3.2	100.6 $\pm$ 5.8

Values are the means  $\pm$  standard deviation (n = 4).

YBU: yoghurt with unadapted *B. bifidum*; YBA: yoghurt with stress-adapted *B. bifidum*;

YRU: yoghurt with unadapted *B. breve*; YRA: yoghurt with stress-adapted *B. breve*;

YAU: yoghurt with unadapted *B. animalis*; YAA: yoghurt with stress-adapted *B. animalis*.

**Table 6.7** Effect of stress adaptation on the syneresis (%) over the 28-day cold storage (4 °C) period of yoghurt containing *B. bifidum*, *B. breve*, and *B. animalis*.

Time (day)	% Syneresis					
	YBU	YBA	YRU	YRA	YAU	YAA
0	34 ± 1.3	33 ± 3.1	32 ± 4.3	38 ± 1.2	37 ± 1.7	36 ± 2.0
7	34 ± 2.9	38 ± 9.5	30 ± 5.1	37 ± 5.3	32 ± 4.5	41 ± 2.6
14	34 ± 2.3	39 ± 4.8	35 ± 1.8	37 ± 3.4	32 ± 3.1	40 ± 0.8
21	33 ± 2.0	41 ± 3.2	34 ± 3.9	38 ± 3.5	36 ± 4.8	42 ± 2.8
28	36 ± 1.4	42 ± 1.5	39 ± 3.5	34 ± 12.0	39 ± 4.2	46 ± 1.6

Values are the means ± standard deviation (*n* = 4).

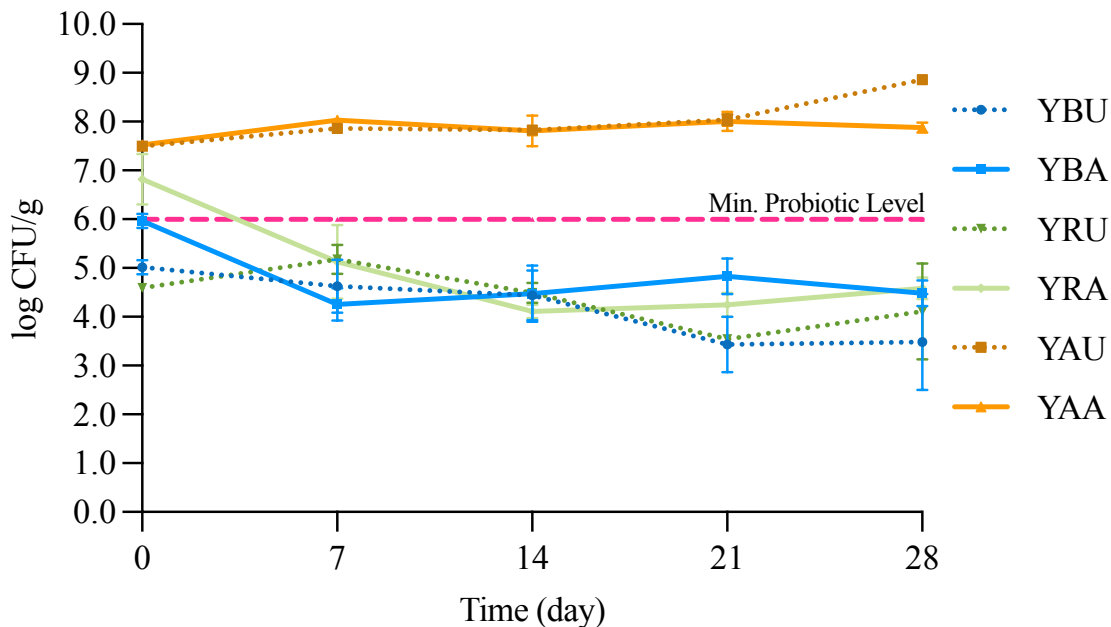
YBU: yoghurt with unadapted *B. bifidum*; YBA: yoghurt with stress adapted *B. bifidum*; YRU: yoghurt with unadapted *B. breve*; YRA: yoghurt with stress-adapted *B. breve*; YAU: yoghurt with unadapted *B. animalis*; YAA: yoghurt with stress-adapted *B. animalis*.

**Table 6.7** Effect of stress adaptation on the syneresis (%) over the 28-day cold storage (4 °C) period of yoghurt containing *B. bifidum*, *B. breve*, and *B. animalis*.

Time (day)	% Syneresis					
	YBU	YBA	YRU	YRA	YAU	YAA
0	34 ± 1.3	33 ± 3.1	32 ± 4.3	38 ± 1.2	37 ± 1.7	36 ± 2.0
7	34 ± 2.9	38 ± 9.5	30 ± 5.1	37 ± 5.3	32 ± 4.5	41 ± 2.6
14	34 ± 2.3	39 ± 4.8	35 ± 1.8	37 ± 3.4	32 ± 3.1	40 ± 0.8
21	33 ± 2.0	41 ± 3.2	34 ± 3.9	38 ± 3.5	36 ± 4.8	42 ± 2.8
28	36 ± 1.4	42 ± 1.5	39 ± 3.5	34 ± 12.0	39 ± 4.2	46 ± 1.6

Values are the means ± standard deviation (*n* = 4).

YBU: yoghurt with unadapted *B. bifidum*; YBA: yoghurt with stress adapted *B. bifidum*; YRU: yoghurt with unadapted *B. breve*; YRA: yoghurt with stress-adapted *B. breve*; YAU: yoghurt with unadapted *B. animalis*; YAA: yoghurt with stress-adapted *B. animalis*.

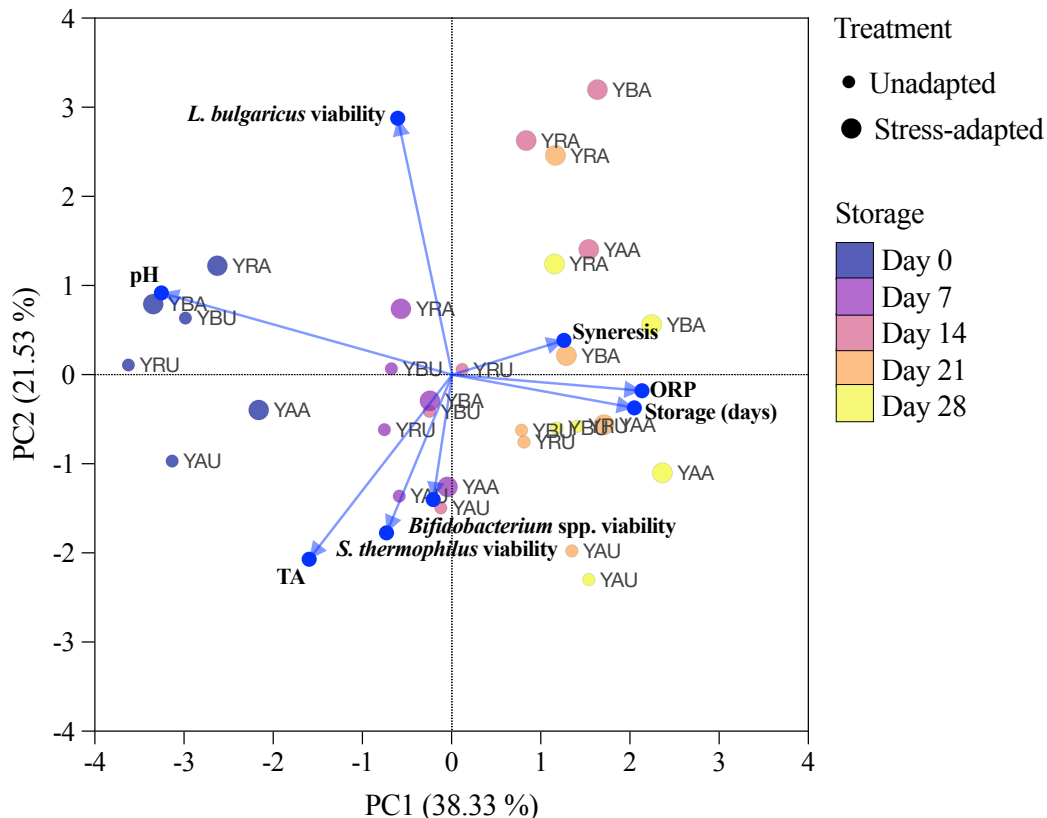


**Figure 6.4** Effect of stress adaptation on probiotic viability in yoghurt containing (A) *B. bifidum*, (B) *B. breve*, and (C) *B. animalis* over the 28-day cold storage (4 °C) period. Values are the means  $\pm$  standard deviation ( $n = 4$ ). YBU: yoghurt with unadapted *B. bifidum*; YBA: yoghurt with stress-adapted *B. bifidum*; YRU: yoghurt with unadapted *B. breve*; YRA: yoghurt with stress-adapted *B. breve*; YAU: yoghurt with unadapted *B. animalis*; YAA: yoghurt with stress-adapted *B. animalis*.

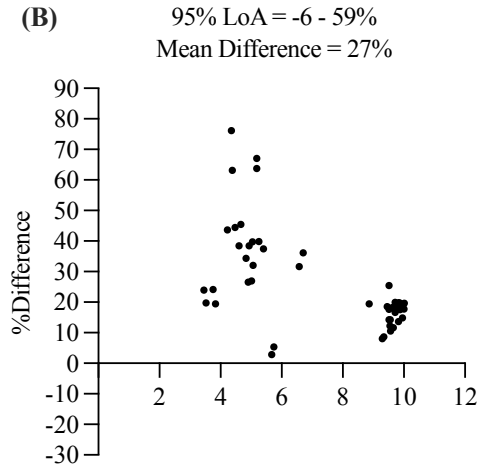
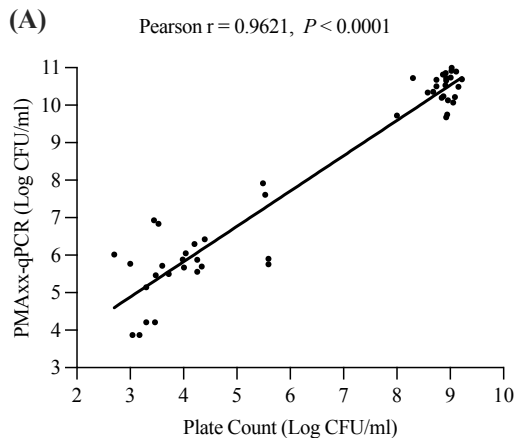
**Table 6.9** The effect of stress adaptation on the viability proportion index (VPI) of *Bifidobacterium* spp. in yoghurt at the end of storage (day 28), as determined by MRS-NNLP.

Species	<i>B. bifidum</i>			<i>B. breve</i>			<i>B. animalis</i>		
Unadapted	0.698	±	0.211	0.895	±	0.213	1.182	±	0.019
Stress-adapted	0.752	±	0.050	0.675	±	0.062	1.047	±	0.012

Values are the means ± standard deviation ( $n = 4$ ).

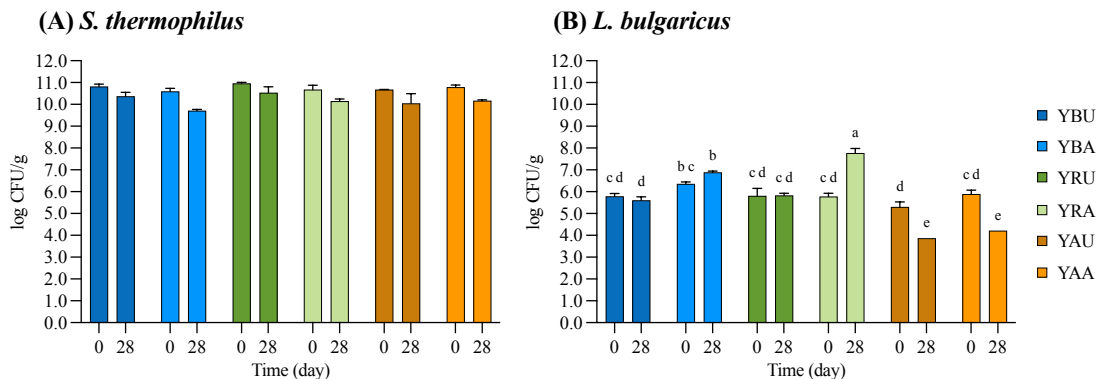


**Figure 6.5** Principal component analysis (PCA) of physicochemical characteristics (pH, titratable acidity (TA), oxidation-reduction potential (ORP)) and viability of yoghurt bacteria (*S. thermophilus* and *L. bulgaricus*) and unadapted or stress-adapted *Bifidobacterium* spp. in yoghurt during refrigerated storage (Days 0, 7, 14, 21 and 28; 4 °C). Percentage of variance of PC1 and PC2 are indicated in parentheses. Arrows indicate the contribution of each variable to PC1 and PC2. YBU: yoghurt with unadapted *B. bifidum*; YBA: yoghurt with stress-adapted *B. bifidum*; YRU: yoghurt with unadapted *B. breve*; YRA: yoghurt with stress-adapted *B. breve*; YAU: yoghurt with unadapted *B. animalis*; YAA: yoghurt with stress-adapted *B. animalis*.



**Figure 6.8** (A) Simple linear regression and (B) Bland-Altman method of comparison (% Difference vs. Average) of the PMAxx-qPCR method compared to the standard plate count method ( $n = 48$ ). The Bland-Altman comparison = expressed as a percentage relative difference [ $100 \times (\text{PMAxx-qPCR count} - \text{Plate count})/\text{average}$ ] vs. average. LoA: Limits of Agreement.

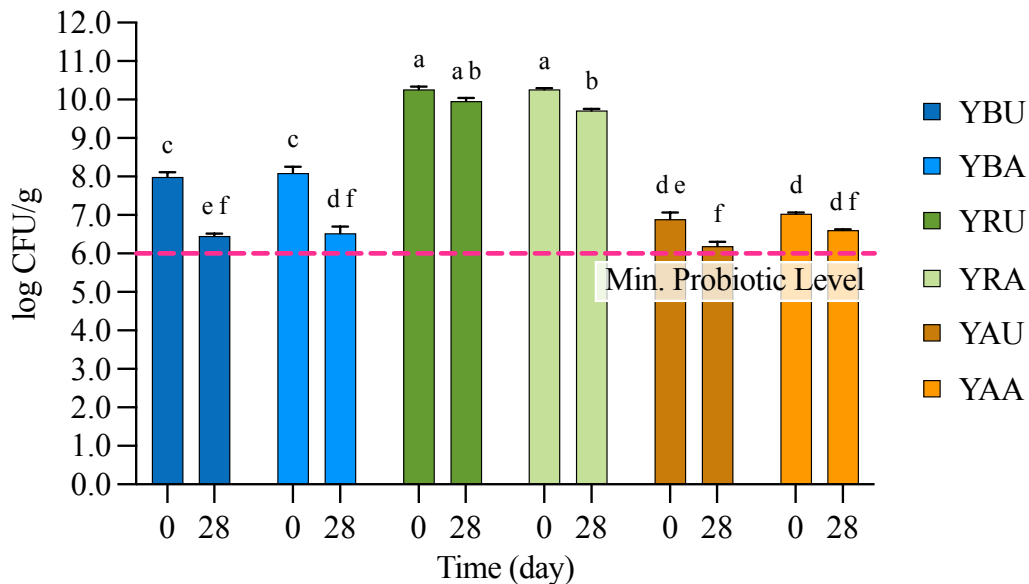




**Figure 6.9** Viability of *S. thermophilus* (A) and *L. bulgaricus* (B) in yoghurt prepared with unadapted or stress-adapted *B. bifidum*, *B. breve* or *B. animalis*, on day 0 and 28 of refrigerated storage (4 °C), as determined by PMAxx-qPCR. Values are the means  $\pm$  standard deviation ( $n = 2$ ). YBU: yoghurt with unadapted *B. bifidum*; YBA: yoghurt with stress-adapted *B. bifidum*; YRU: yoghurt with unadapted *B. breve*; YRA: yoghurt with stress-adapted *B. breve*; YAU: yoghurt with unadapted *B. animalis*; YAA: yoghurt with stress-adapted *B. animalis*.

**Table 6.10** Analysis of variance showing the effect of bacterial species and adaptation treatment on the viability of unadapted or adapted *Bifidobacterium* spp. and resulting VPI<sup>1</sup>, as measured by PMAxx-qPCR, in yoghurt during 28 days of refrigerated storage.

Source of Variation	DF	<i>P-Value</i>	
		PMAxx-qPCR	VPI <sub>28</sub>
<b>Main Effects</b>			
<i>Bifidobacterium</i> spp. ( <i>B. bifidum</i> , <i>B. breve</i> , <i>B. animalis</i> )	2	< 0.001	< 0.001
Stress Adaptation (Unadapted, Adapted)	1	< 0.001	-
<b>Interactions</b>			
Spp. x Stress Adaptation	2	< 0.001	-
Spp. x Shelf-life	2	0.013	-



**Figure 6.10** Probiotic viability in yoghurt prepared with unadapted or stress-adapted *B. bifidum*, *B. breve* or *B. animalis*, on day 0 and 28 of refrigerated storage (4 °C), as determined by PMAxx-qPCR. Values are the means  $\pm$  standard deviation ( $n = 2$ ). YBU: yoghurt with unadapted *B. bifidum*; YBA: yoghurt with stress-adapted *B. bifidum*; YRU: yoghurt with unadapted *B. breve*; YRA: yoghurt with stress-adapted *B. breve*; YAU: yoghurt with unadapted *B. animalis*; YAA: yoghurt with stress-adapted *B. animalis*.

**Table 6.11** The effect of stress adaptation on the viability proportion index (VPI) of *Bifidobacterium* spp. in yoghurt at the end of storage (day 28), as determined by PMAxx-qPCR.

Species	<i>B. bifidum</i>			<i>B. breve</i>			<i>B. animalis</i>		
Unadapted	0.808	±	0.004	0.971	±	0.015	0.900	±	0.037
Stress-adapted	0.806	±	0.006	0.947	±	0.002	0.940	±	0.007

Values are the means ± standard deviation ( $n = 4$ ).

**Table 6.12** Effect of stress adaptation on the predicted shelf-life of probiotic yoghurt containing *B. bifidum*, *B. breve* or *B. animalis*, based on the recommended minimum viable level for probiotics in yoghurt.

<i>Bifidobacterium</i> spp. in yoghurt	Shelf-life based on MRS-NNLP (days)		Shelf-life based on PMAxx-qPCR (days)	
	Unadapted	Stress Adapted	Unadapted	Stress Adapted
<i>B. bifidum</i>	0	< 7	28	28
<i>B. breve</i>	0	< 7	28	28
<i>B. animalis</i>	28	28	28	28

<sup>1</sup> Shelf-life was established based on the minimum viable count requirement of 6 log CFU/g, as mandated by CODEX STAN 243-2003 (FAO/WHO, 2002). This criterion applies to microorganisms that are added as supplementary cultures to yoghurt dairy products, beyond the primary starter culture, whereby a content claim can be made in the labelling.