

Supplementary Data

Table S1. Gene copy number of 16s rRNA and *tuf* genes for the five target reference species.

Data Source	Organism	Strain Number	Copies	
			16S rRNA	<i>tuf</i>
ATCC website (genomes.atcc.org)	<i>B. bifidum</i>	ATCC 11863	3	1
ATCC website (genomes.atcc.org)	<i>B. breve</i>	ATCC 15700	3	1
NCBI, Genbank CR954253.1	<i>L. delbrueckii subsp. bulgaricus</i>	ATCC 11842	9	1
ATCC website (genomes.atcc.org)	<i>S. thermophilus</i>	ATCC 19258	5	1
NCBI, Genbank FM179322.1	<i>L. rhamnosus</i>	ATCC 53103	5	1

Table S2. Identity Matrix (%) of *tuf* gene sequences

Organism	Strain	Identity (%)				Overall Identity (%)	Overall Variation (%)
		ATCC 29521	ATCC 19258	ATCC 11842	ATCC 53103		
<i>Bifidobacterium bifidum</i>	ATCC 29521	100.00	63.83	66.29	68.24		
<i>Streptococcus thermophilus</i>	ATCC 19258	63.83	100.00	71.31	71.26	69.31	30.7
<i>Lactobacillus delbrueckii subsp. bulgaricus</i>	ATCC 11842	66.29	71.31	100.00	74.90		
<i>Lactocaseibacillus rhamnosus</i>	ATCC 53103	68.24	71.26	74.90	100.00		

Analysed using Multiple Sequence Comparison by Log Expectation (MUSCLE) program (<https://www.ebi.ac.uk/Tools/msa/muscle/>).

Table S3. Identity Matrix (%) of 16S rRNA gene sequences

Organism	Strain	Identity (%)				Overall Identity (%)	Overall Variation (%)
		ATCC 29521	ATCC 19258	ATCC 11842	ATCC 53103		
<i>Bifidobacterium bifidum</i>	ATCC 29521	100.00	77.20	78.28	79.16		
<i>Streptococcus thermophilus</i>	ATCC 19258	77.20	100.00	83.37	84.62	81.73	18.27
<i>Lactobacillus delbrueckii subsp. bulgaricus</i>	ATCC 11842	78.28	83.37	100.00	87.73		
<i>Lacticaseibacillus rhamnosus</i>	ATCC 53103	79.16	84.62	87.73	100.00		

Analysed using Multiple Sequence Comparison by Log Expectation (MUSCLE) program (<https://www.ebi.ac.uk/Tools/msa/muscle/>).

Table S4. Real-Time qPCR Primer Properties

Primer Name	Primer Length (bp)	Amplicon Size (bp)	Hairpin 3' end (Kcal/mol)	Primer T _m (°C)	GC (%)	Self complementarity	Self 3' Complementarity
ST-F	23	118	1.46	60.00	43.48	4.00	2.00
ST-R	23		-0.50	59.56	43.48	6.00	6.00
Bb-1F	21	149	-0.27	60.20	52.38	3.00	3.00
Bb-1R	20		-0.27	60.04	60.00	3.00	1.00
Ldb-1F	22	121	-1.26	60.22	50.00	5.00	2.00
Ldb-1R	22		0.97	59.51	50.00	2.00	2.00
Lcr-1F	22	117	0.97	59.97	50.00	6.00	0.00
Lcr-1R	22		0.97	59.76	50.00	6.00	1.00

Legend: T_m – Melt Temperature

1	CGTGGACTGC	CCGGGCCACG	CCGACTTCGT	GAAGAACATG	ATCACCGGTG
51	CTGCCAGAT	GGATGGCGCC	ATCCTCGTTG	TGGCCGCCAC	CGACGGCCCG
101	ATGGCCAGA	CCC GCAGCA	CGTTCTGCTC	GCCCCCAGG	TGGGTGTCCC
151	GCGTATCCTC	GTCGCCCTGA	ACAAGTGC GA	CATGGTCGAG	GACGAGGAGC
201	TCATCGAGCT	CGTTGAGGAA	GAGGTCCGTG	ACCTCCTCGA	CGAGAACGGC
251	TTCGACCGCG	ACTGCCCGGT	CATCCGTACC	TCCGCCTACG	GCGCTCTGCA
301	CGATGACGCT	CCG GACCAG	ACAAGTGGGT	CCAGACCGTC	AAGGACCTGA
351	TGGACGCCGT	CGACGAGTAC	ATCCCCACCC	CGGTTACGA	CCTGGACAAG
401	CCGTTCCCTGA	TGCCTATCGA	GGACGTCTTC	ACCATCTCCG	GCCGTGGTAC
451	CGTCGTACC	GGTCGTGTTG	AGCGTGGCCA	GCTGGCCGTC	AACACCCCGG
501	TCGAGATCGT	CGGCATCCGC	CCGACCAGAG	CCACCACCGT	TACCTCCATC
551	GAGACGTTCC	ACAAGACCAT	GGACGCCTGC	GAGGTGGCG	ACAACACCGG
601	TCTGCTGCTC	CGCGGCATCA	ACCGTACGGA	CGTCGAGCGT	GGTCAGGTTG
651	TGGCCAAGCC	GGGCTCCGTC	ACCCGCACA	CCAAGTTCGA	GGGCGAAGTC
701	TACGTGCTGA	CCAAGACGAG	GGCGGCCGTC	ACTCGCCGTT	CTTCTCCAAC
751	TACCGTCCGC	AGTCTACT			

B

1	CTTCAGGCCG	TATCGACCGT	GGTACTGTTA	AGGTTGGCGA	CAGCGTTGAA
51	ATCGTTGGTT	TGGTAGAAAA	GGTCTTGACT	TCAGTTGTAA	CTGGCTTGGA
101	AATGTTCCAC	AAGACTCTTG	ACTTGGGTGA	AGCCGGCGAC	AACGTTGGTG
151	TATTGCTCCG	TGGTGTGAC	CGTGACAAA	TCGTTCTGG	CCAAGTTTTG
201	GCAGCTCCAG	GCTCAATCAA	GACCCACAGA	ACTTTCAAGG	GTCAAGTCTA
251	CATCTTGAGT	AAGGAAGAAG	GT		

C

1	CTTCAGGTCG	TATCGATCGT	GGTACGGTTA	AGGTCGGCGA	TGAAGTTGAA
51	ATCATCGGTT	TGAAGCCAGA	TGTTCTCAAG	TCCACCGTTA	CCGGTCTTGA
101	AATGTTCCGT	AAGACCTTGG	ATCTTGGTGA	AGCCGGCGAT	AACGTTGGTG
151	TCCTGCTTCG	TGGGATCAAC	CGTGACCAAG	TTGAACGTGG	CCAAGTTTTG
201	GCAAAGCCAG	GTTCAATCCA	ATTGCACAAC	AAGTTCAAGG	GTGAAGTTTA
251	TATCTTGACA	AAAGAAGAAG	GT		

Figure S1. Designed primer sets (Yellow-Forward Primer & Purple-Reverse Primer) flanking target regions on the *tuf* genes of *B. bifidum* (A), *L. delbrueckii* (B) and *L. rhamnosus* (C). Primers were designed using the online software primer 3 plus (<https://www.primer3plus.com/>).

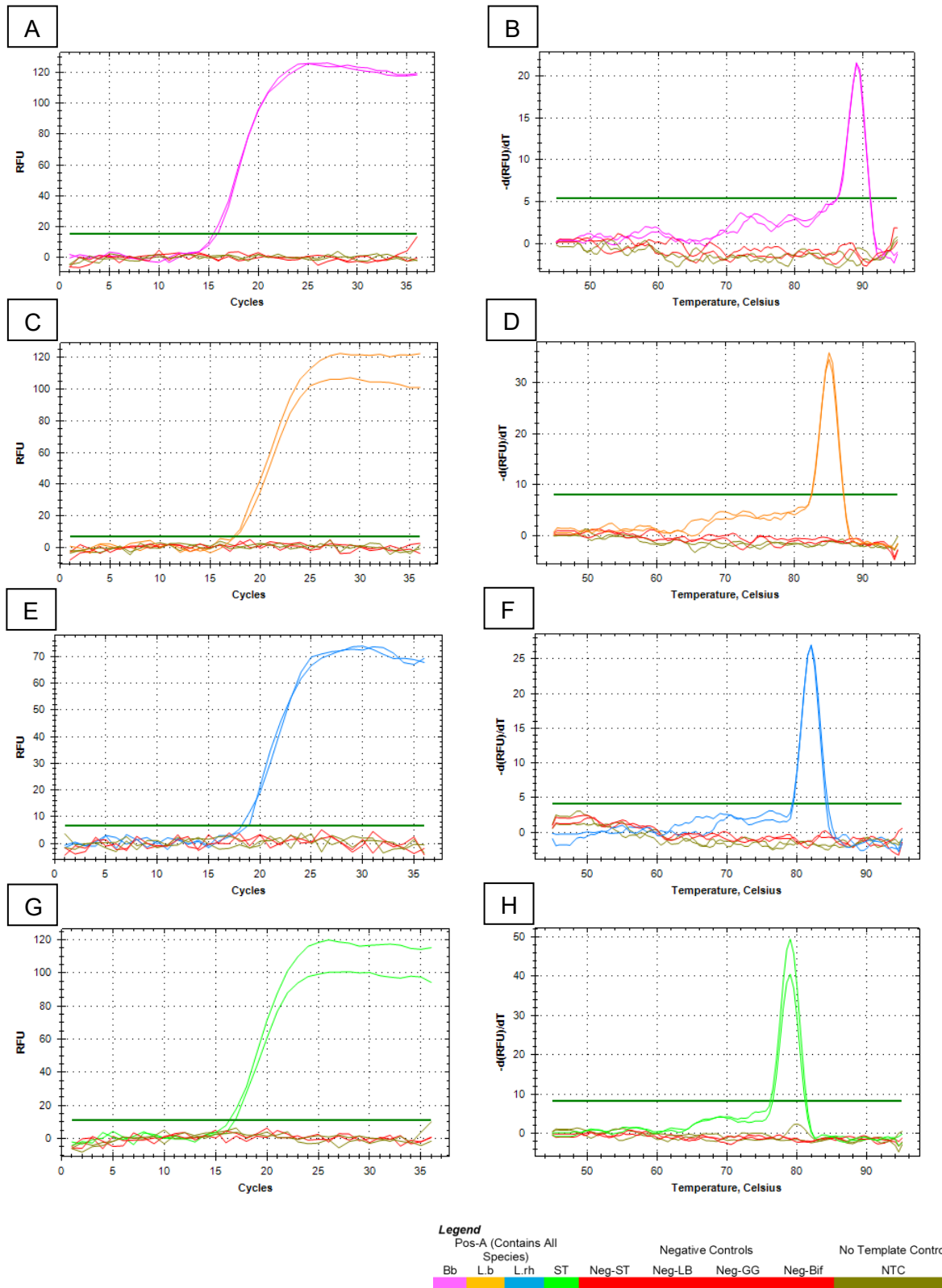


Figure S2. Amplification (left) and melt (right) curves showing the specificity of the four sets of primers in mixed species environment. Graphs A-B represented primers for *B. bifidum* (Bb), C-D for *L. delbrueckii* subsp. *bulgaricus* (L.b), E-F for *L. rhamnosus* (L.rh), and G-H for *S. thermophilus* (ST).

Standard Curves: Limit of Quantification (LOQ)

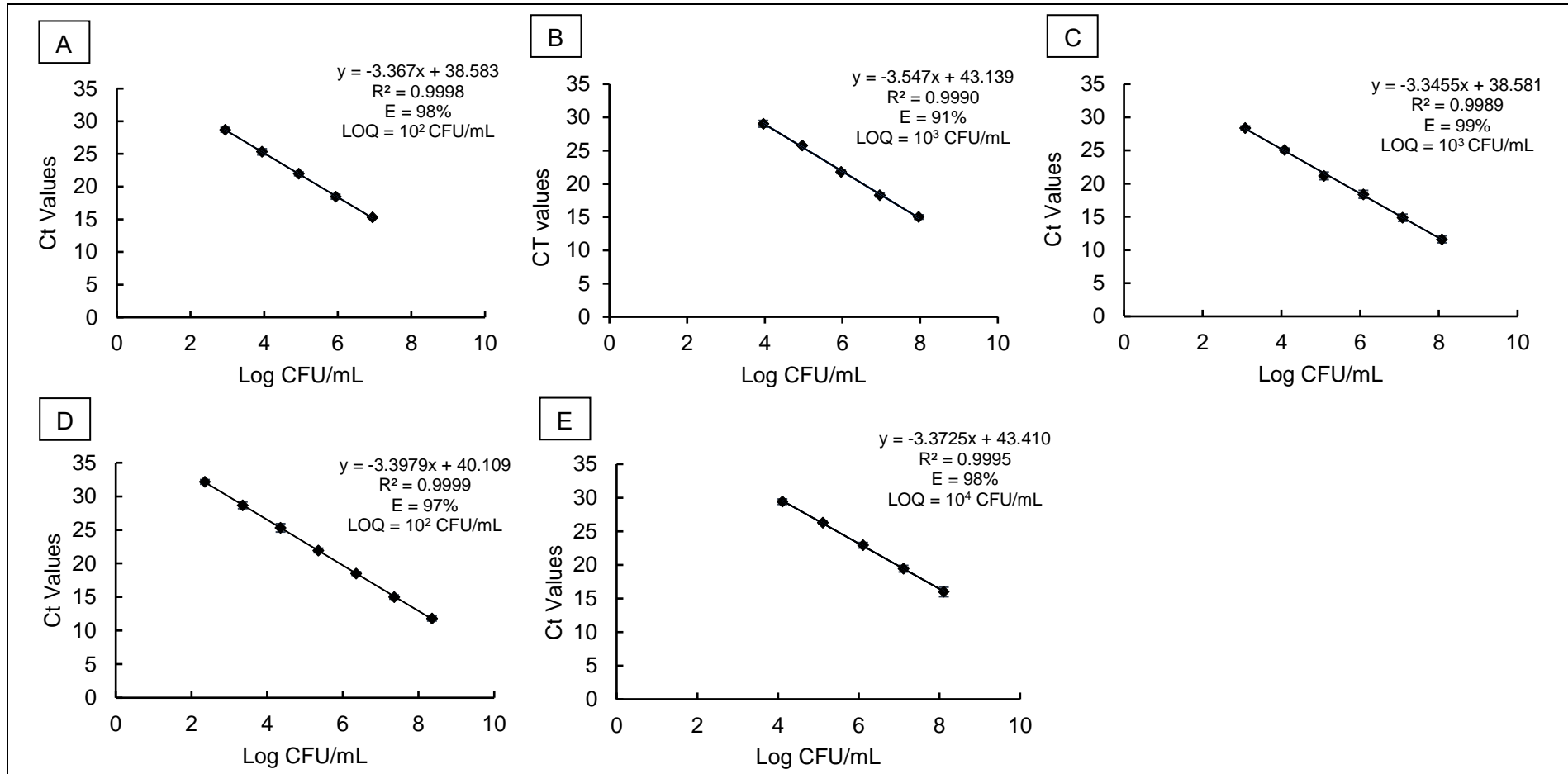


Figure S3. Standard curves of PMAxTM-qPCR assay created and used for determining the limit of quantification (LOQ) for *B. bifidum* ATCC 11863 (A), *B. breve* ATCC 15700 (B), *L. delbrueckii* subsp. *bulgaricus* (C), *S. thermophilus* (D), and *L. rhamnosus* (E). Each point represents the mean \pm standard deviation of CT values of two independent runs ($n = 4$). Each run was carried out in duplicate