

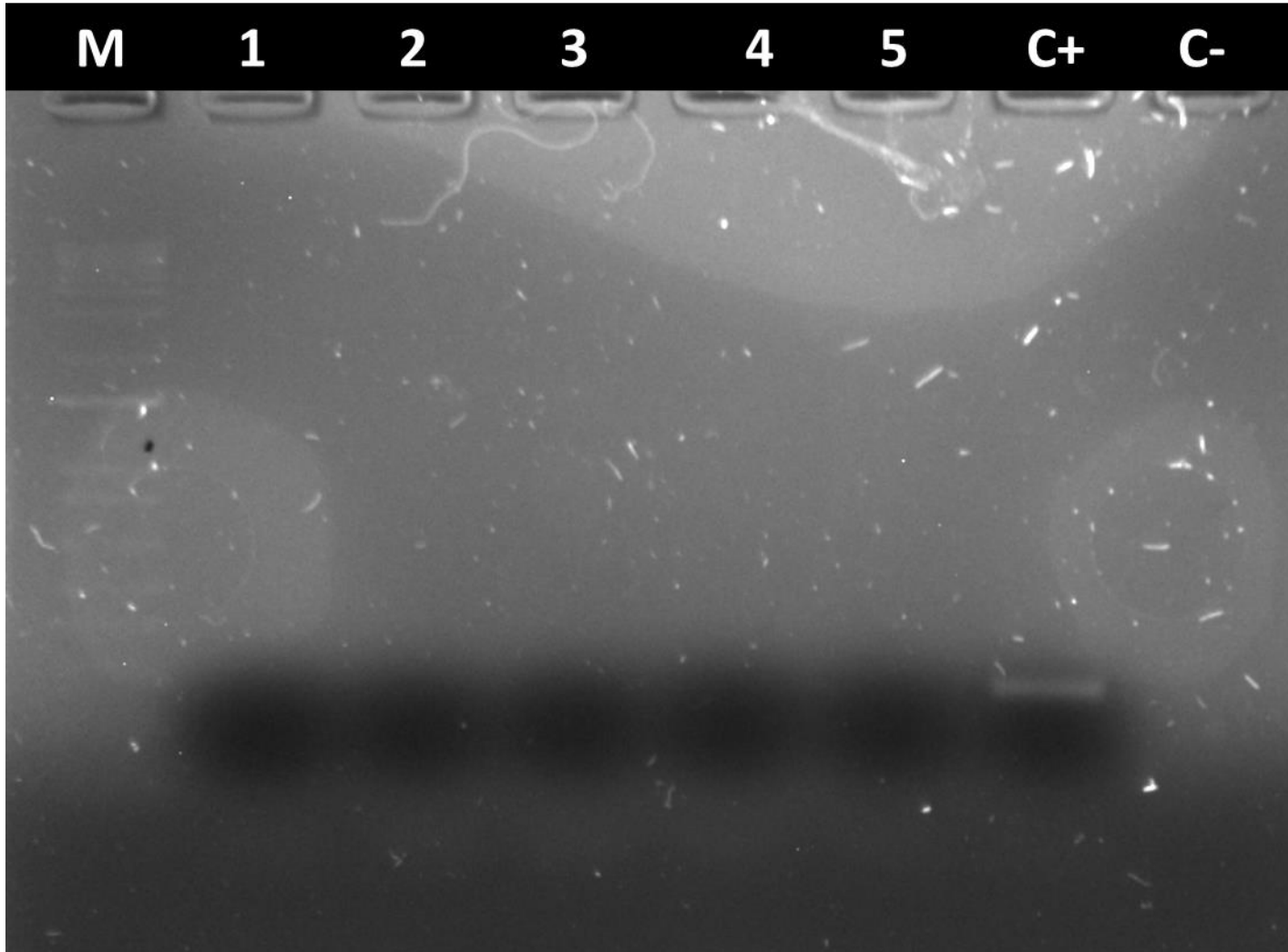
# Supplementary figure S1

## **Improvement and validation of a genomic DNA extraction method for human breastmilk**

Methods and Protocols, MDPI

## Test to amplify V3-V4 fragment from GTC extractions

A1



Five samples were randomly selected from extraction #1 of human milk samples

Agarose gel electrophoresis:

80 Vots, 46 min

4% agarose gel

Expected DNA-amplicón ~400 pb

Forward: 341F: 5'-CCTACGGGNGGCWGCAG-3'

Reverse: 785R: 5'-GGACTACHVGGGTATCTAATCC-3'

M = 1kb plus (Invitrogen)

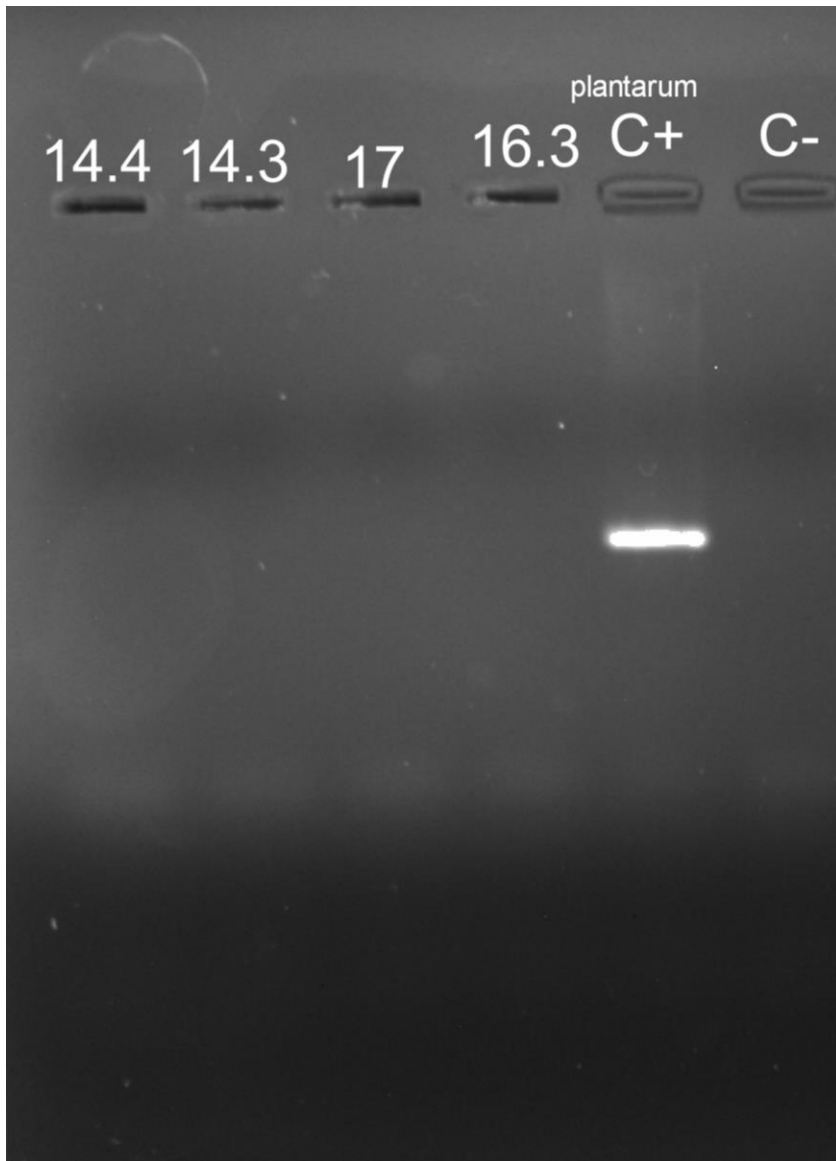
1-5 = Random samples from first extraction

C+ = DNA extracted from bacteria culture with GTC method

C- = Amplification without gDNA

## Test to amplify V3-V4 fragment from GTC extractions

A2



Samples randomly selected from all extraction attempts

14.4 Sample #14 GTC method, attempt 4

14.3 Sample # 14 GTC method, attempt 3

17 Sample # 17 GTC method, attempt 1

16.3 Sample # 16 GTC method, attempt 3

C+ Fresh culture from *Lactobacillus plantarum* GTC method

C- Amplification without gDNA

Agarose gel electrophoresis:

80 Vots, 46 min

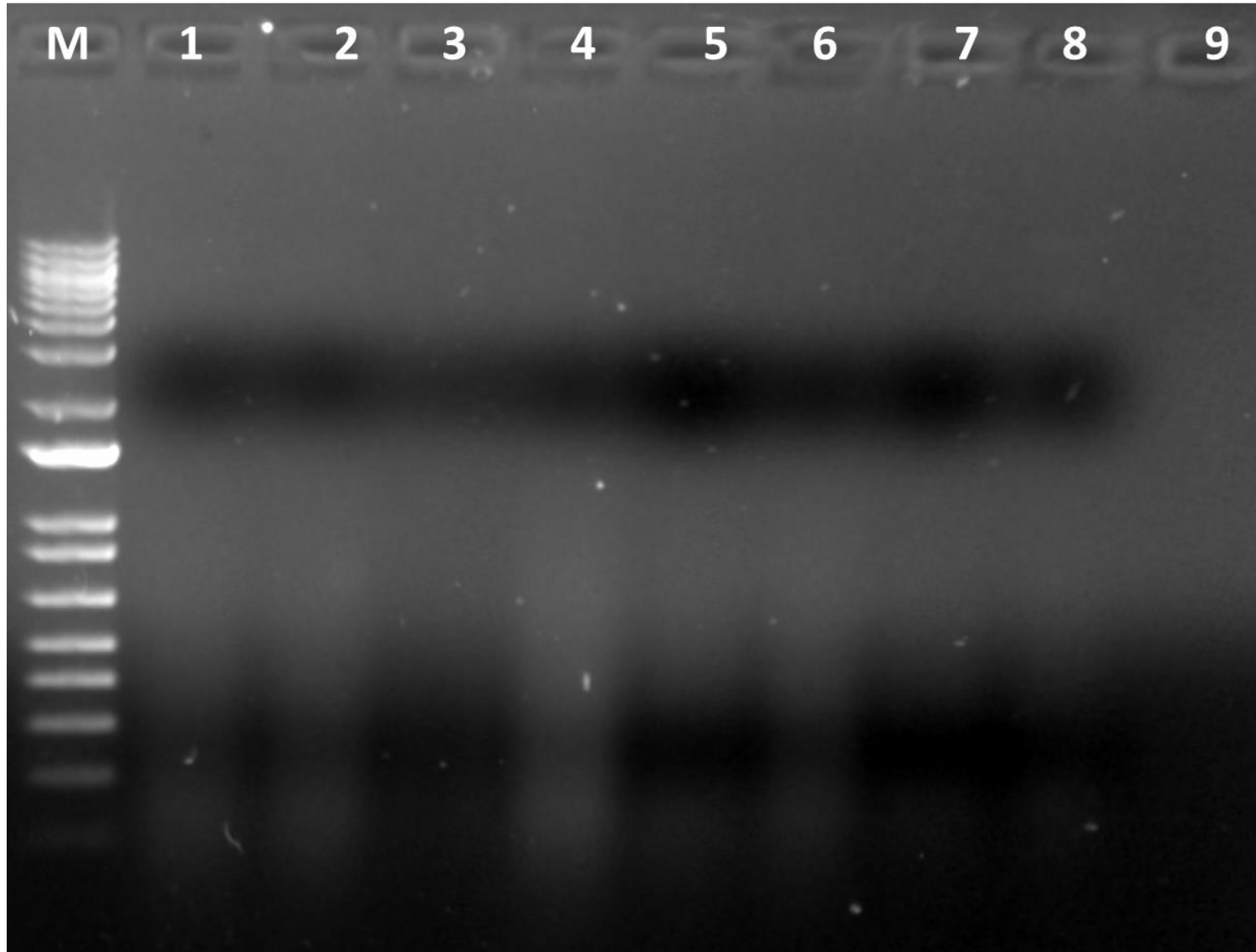
4% agarose gel

Expected DNA-amplicón ~400 pb

Forward: 341F: 5'-CCTACGGGNGGCWGCAG-3'

Reverse: 785R: 5'-GGACTACHVGGGTATCTAATCC-'3

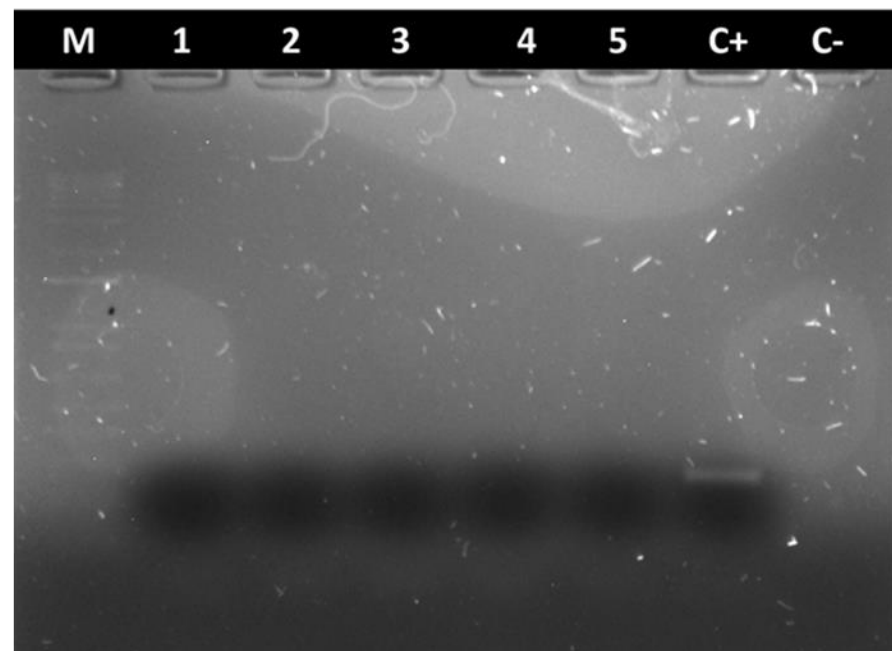
## Test to amplify ITS1 fragment from GTC extractions



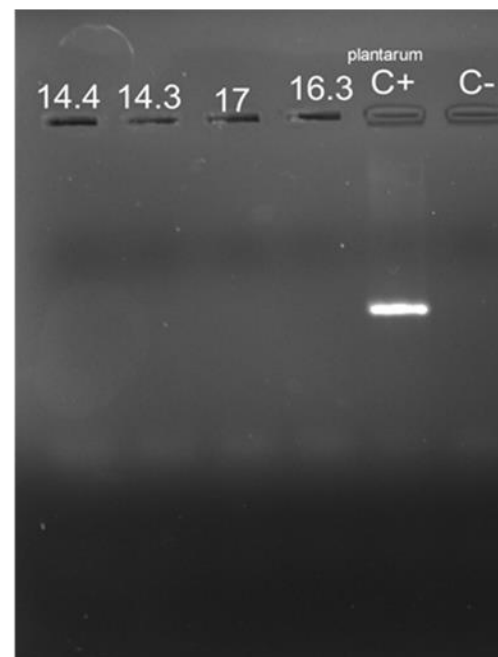
Samples randomly selected  
from the first extraction

Agarose gel electrophoresis:  
80 Volts, 46 min  
4% agarose gel  
Expected DNA-amplicón ~200 pb  
Forward: ITS-1: 5'-  
TCCGTAGGTGAACCTGCGG-3'  
Reverse: ITS-2: 5'-  
GCTGCGTTCTTCATCGATGC-3'  
1-9 Samples were randomly selected  
to show ITS-1 amplicons

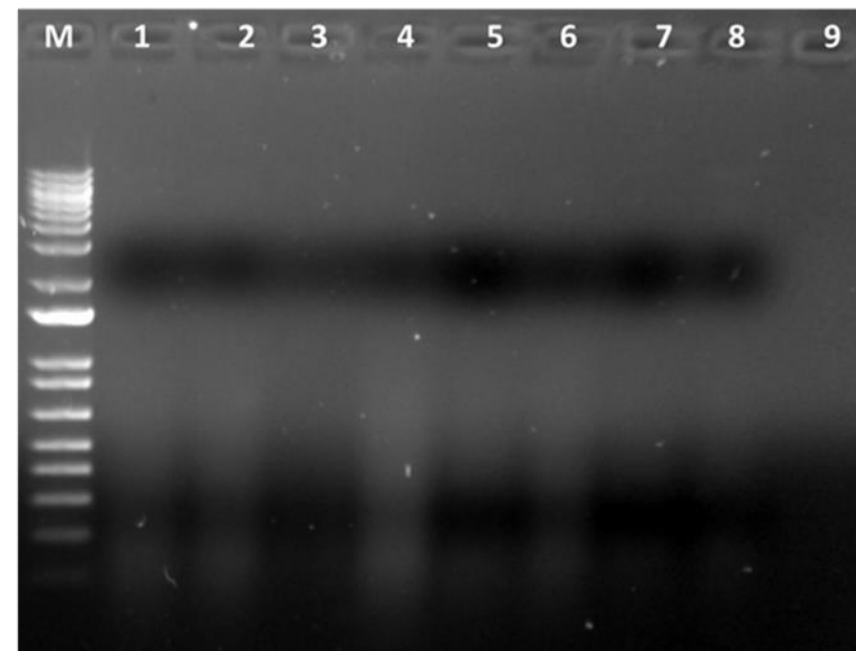
(a)



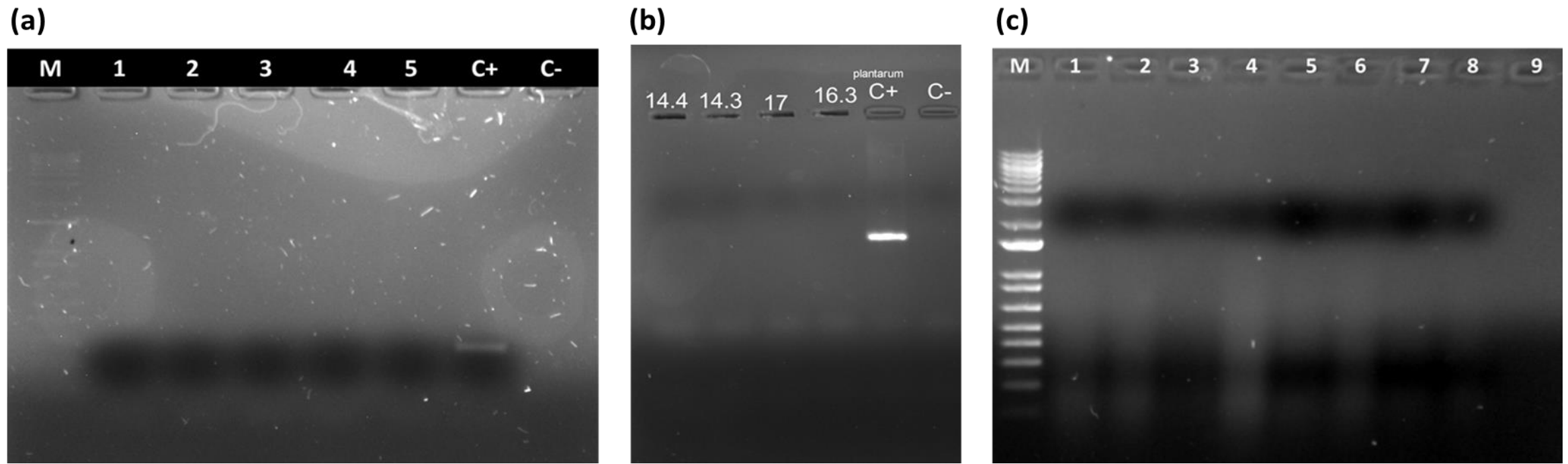
(b)



(c)



**Supplementary Figure S1.** The figure shows representative results from gel electrophoresis analysis of PCR-amplicons obtained from human milk DNA samples using the GTC method. Panel (a) displays the results of the V3-V4 DNA-amplicon test, where wells 1 to 5 contain samples randomly selected from the first extraction. The positive control (C+) shows amplification of the V3-V4 fragment of *Lactobacillus plantarum* using the GTC method, while the negative control (C-) shows amplification without gDNA. Panel (b), presents results from the V3-V4 DNA-amplicon test with samples marked as 14.4, 14.3, 17, and 16.3, which were randomly selected from different gDNA extraction attempts to test the V3-V4 amplification. The positive control (C+) shows amplification of the V3-V4 fragment of *Lactobacillus plantarum* using the GTC method, while the negative control (C-) shows amplification without gDNA. Panel (c) shows the results of the ITS-1 DNA-amplicon test, where wells 1 to 9 contain samples randomly selected from the first extraction. Finally, a gene ruler DNA ladder (1Kb plus from Invitrogen™, MA, USA) is included in all panels and denoted by M.



**Figure S1.** The figure shows representative results from agarose gel electrophoresis analysis of PCR-amplicons obtained from human milk DNA samples using the GTC method. Panel (a) displays the results of the V3-V4 DNA-amplicon test, where wells 1 to 5 contain samples randomly selected from the first extraction. The positive control (C+) shows amplification of the V3-V4 fragment of *Lactobacillus plantarum* using the GTC method, while the negative control (C-) shows amplification without gDNA. Panel (b), presents results from the V3-V4 DNA-amplicon test with samples marked as 14.4, 14.3, 17, and 16.3, which were randomly selected from different gDNA extraction attempts to test the V3-V4 amplification. The positive control (C+) shows amplification of the V3-V4 fragment of *Lactobacillus plantarum* using the GTC method, while the negative control (C-) shows amplification without gDNA. Panel (c) shows the results of the ITS-1 DNA-amplicon test, where wells 1 to 9 contain samples randomly selected from the first extraction. Finally, a gene ruler DNA ladder (1Kb plus from Invitrogen™, MA, USA) is included in all panels and denoted by M.