ABSTRACT

ASILO, EDEN BETH B. University of the Philippines Los Baños, June 2018, **Optimization of Functionalized Magnetic Nanoparticles Use for Polymerase Chain Reaction (PCR)-Based Detection of Bacterial Contaminants in Ethanol Fermentation Using *Lactobacillus fermentum* Beijerinck as Model Organism.**

Major Professor: Dr. FRANCISCO B. ELEGADO

Functionalized magnetic nanoparticle (MNP-F1) was used in tandem with PCR to detect lactic acid bacterial contamination in ethanol fermentation. It was revealed that the percent cell capture efficiency (%CCE) by MNP-F1 of 5 $mgmL^{-1}$ of *Lactobacillus fermentum* at an initial population of 10^8 $CFUmL^{-1}$ in MRS broth cultures ranged from 99.61% to 99.23% with respect to strain variability. %CCE was comparable even at ten (0.5 $mgmL^{-1}$) and 100 (0.05 $mgmL^{-1}$) fold decrease of MNP-F1 concentration which ranged from 94.74±0.16 to 99.30±0.3. Significantly, %CCE increased at lower MNP-F1 concentration which pre-concentrated 10^8 $CFUmL^{-1}$ using 0.0125 $mgmL^{-1}$ MNP-F1 with %CCE of 99.70±0.3. Furthermore, Transmission Electron Microscopy successfully validated the MNP-F1-*Lb. fermentum* FM7 conjugation of the target cells. MNP-F1 promoted simple and rapid genomic DNA template preparation of captured cells regardless of reagent-less and centrifuge-free gDNA extraction.

Successful detection of *Lb. fermentum* on MRS broth and fermenting molasses matrices with *S. cerevisiae* BIOTECH 2030 in six MNP-F1 concentrations: 0.5, 0.25, 0.10, 0.05, 0.025 and 0.0125 $mgmL^{-1}$ were confirmed through PCR. Interestingly, 10 $CFUmL^{-1}$ cell concentration was also detected using the lowest MNP-F1 concentration of 0.0125 $mgmL^{-1}$confirming MNP-F1 applicability to enhanced PCR detection limit from 10^4 $CFUmL^{-1}$ cells to 10 $CFUmL^{-1}$ cell.

Keywords: Functionalized magnetic nanoparticle, lactic acid bacterial contamination, ethanol fermentation, percent cell capture efficiency, *Lactobacillus fermentum* FM7, *Saccharomyces cerevisiae* BIOTECH 2030

Table 1. Number of CFU mL^{-1} and %CCE of *Lb. fermentum* FM7 against varying MNP-F1 concentrations grown on MRS agar for 24 hours at 37^0C.

MNP-F1 VARYING CONCENTRATIONS ($mgmL^{-1}$)	ORIGINAL EST. VIABLE CELL CONCENTRATION ($CFUmL^{-1}$)	CAPTURED VIABLE CELL CONCENTRATION ($CFUmL^{-1}$)	MEAN %CCE
5.0	0^b 1.08×10^{10a}	0 1.98×10^{10}	--- 99.61
0.5	0^b 1.68×10^{8a}	0 1.19×10^{9}	--- 94.29±0.16
0.25	0^b 1.78×10^{8a}	0 2.68×10^{8}	--- 96.38±0.21
0.10	0^b 1.49×10^{8a}	0 2.82×10^{8}	--- 97.38±0.06
0.05	0^b 1.38×10^{8a}	0 1.21×10^{8}	--- 99.30±0.23
0.025	0^b 2.31×10^{8a}	0 1.19×10^{8}	--- 96.34±0.08
0.0125	0^b 1.81×10^{8a}	0 9.6×10^{8}	--- 99.70±0.35

a = refers to Control 1 (NO MNPs): 450 µL PBS + 50 µL 10^{-5}
b = refers to Control 2 (NO CELLS): 450 µL PBS + 50 µL MNP-F1
MRD pH = 7.03 for Concentrations A, B, C, D while pH = 7.08 for Concentrations E and F.

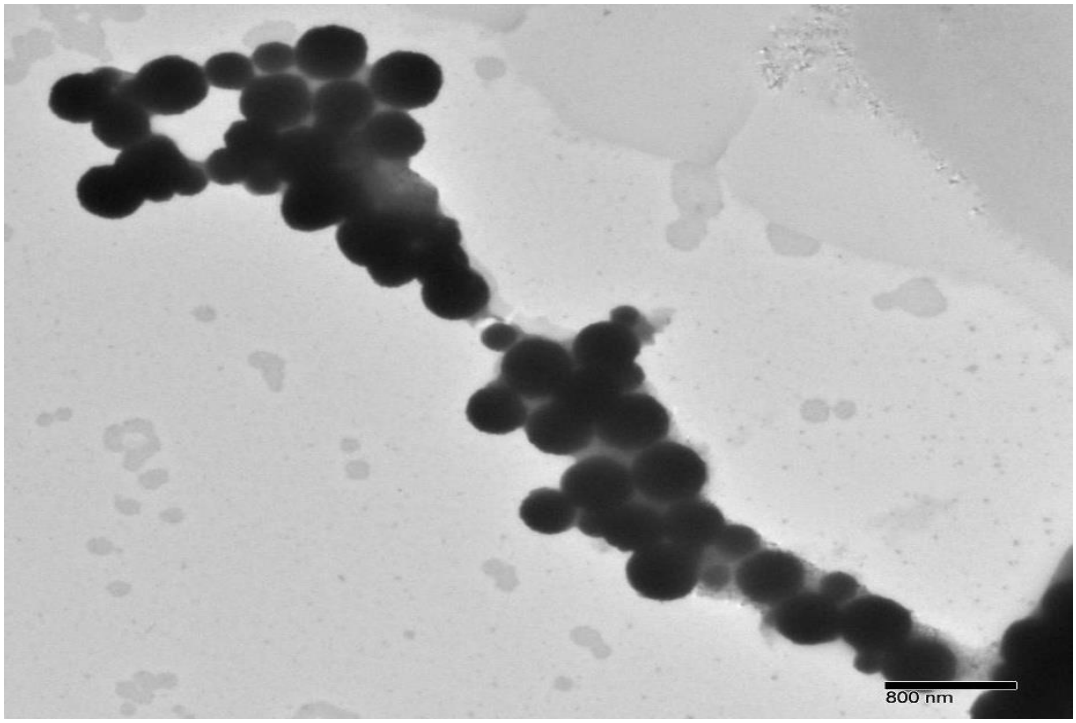


Figure 1. TEM image of MNP-F1-captured *Lb. fermentum* FM7 cell at 10^8 CFUmL^{-1} using 0.5 mgmL^{-1} in MRD (pH = 7.00). MNP-F1 concentration was 0.5 mgmL^{-1} dispersed in HPLC-grade water. *Lb. fermentum* FM7 cells were grown in MRS broth for 10-14 hours at 37^0C prior to magnetic capture assay. Direct Magnification: 3000x at 100.0kV was used for all images using Jeol JEM-1220 Transmission Electron Microscope (JEOL, USA, Inc.). Scale bars represent 1µm at 600 nm. (Courtesy of Mr. Dave B. de Vera, RITM-EM Laboratory, Alabang, Metro Manila, Philippines).

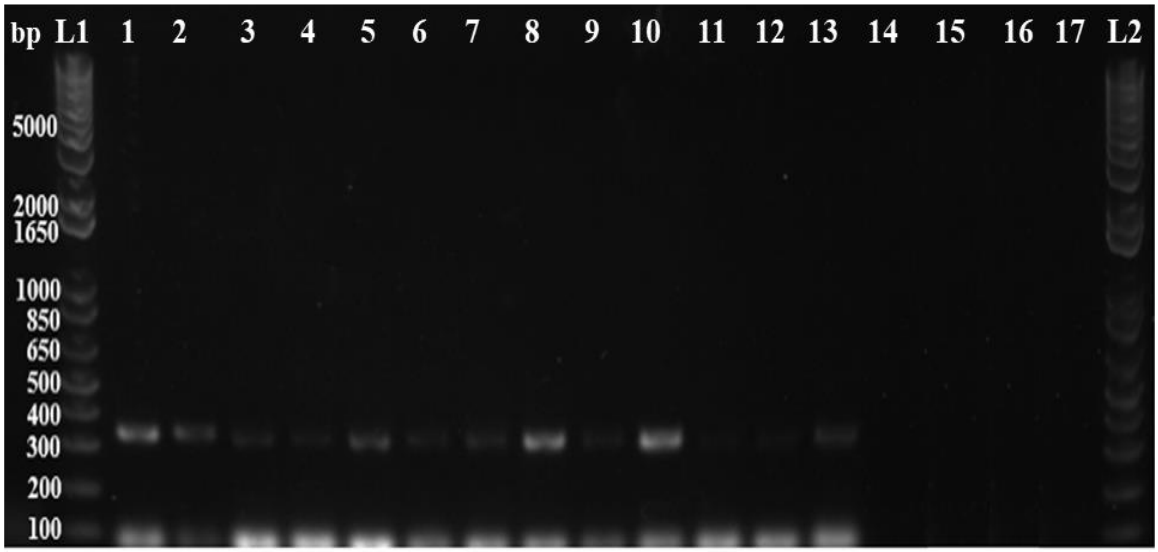


Figure 2. Amplified DNA bands of MNP-F1-captured *Lb. fermentum* FM7 Cells through PCR from MRS broth culture in different cell dilutions on 1% Agarose gel electrophoresis. Stained with GelRed™ run under 100V for 35 mins. Target product size of *Lb. fermentum* is 337 bp. (**A**) L1 and L2 = 1µL 1Kb Plus DNA Ladder (Invitrogen™); all lanes were loaded with 1 µL from 50 µL MNP-F1 + 400 µL MRD with 50 µL cell suspension in: **Lane 1** = 10^{10} CFUmL^{-1}; **Lane 2** = 10^{9} CFUmL^{-1}; **Lane 3** = 10^{8} CFUmL^{-1}; **Lane 4** = 10^{7} CFUmL^{-1}; **Lane 5** = 10^{6} CFUmL^{-1}; **Lane 6** = 10^{5} CFUmL^{-1}; **Lane 7** = 10^{4} CFUmL^{-1}; **Lane 8** = 10^{3} CFUmL^{-1}; **Lane 9** = 10^{2} CFUmL^{-1}; **Lane 10** = 10^{1} CFUmL^{-1}; **Lane 11** = 10^{8} dilution + 400 µL MRD only; **Lane 12** = 10^{6} CFUmL^{-1} + 400 µL MRD only; Control 1: NO MNPs: **Lane 13** = 50 µL 10^{4} CFUmL^{-1} + 450 µL MRD; **Lane 14** = 50 µL 10^{3} CFUmL^{-1} + 450 µL MRD; **Lane 15** = 50 µL 10^{2} CFUmL^{-1} + 450 µL MRD; **Lane 16** = 50 µL 10^{1} CFUmL^{-1} + 450 µL MRD; Control 2: NO CELLS: **Lane 17** = 450 µL MRD + 50 µL MNP-F1. MNP-F1 concentration = 0.05 mgmL^{-1}. *Lb fermentum* FM7 cells were 10 to 14-hour old.

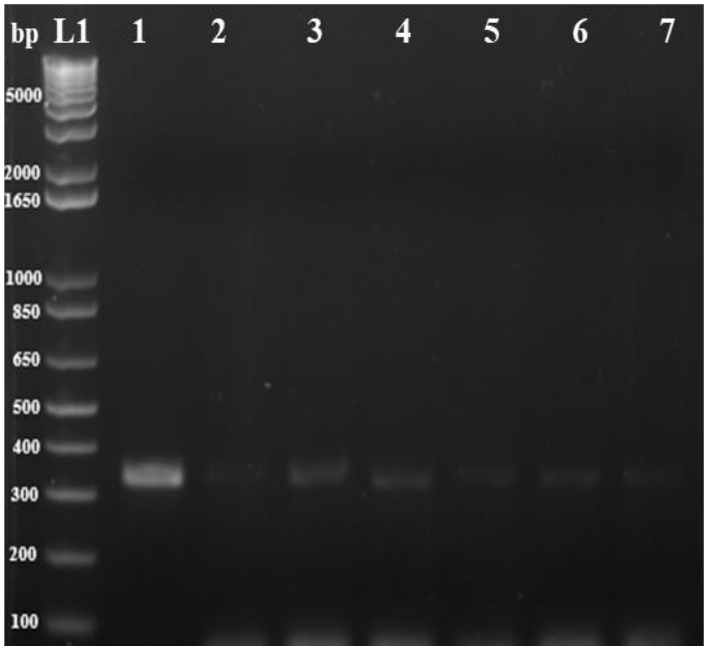


Figure 3. Amplified DNA bands of MNP-F1-captured *Lb. fermentum* FM7 cells through PCR amplification from MRS broth culture in varying MNP-F1 concentrations with 10^4 $CFUmL^{=1}$ cells on 1% Agarose gel electrophoresis. Stained with GelRed™ run under 100V for 40 mins. Target product size of *Lb. fermentum* is 337 bp. (**A**) L = 1µL 1Kb Plus DNA Ladder (Invitrogen™); all lanes were loaded with 1 µL from 50 µL 10^4 $CFUmL^{-1}$ cells + 400 µL MRD with 50 µL of varying MNP-F1 concentrations in: **Lane 1** = gDNA of *Lb. fermentum* FM7 (by CTAB extraction); **Lane 2** = 0.5 $mgmL^{-1}$; **Lane 3** = 0.25 $mgmL^{-1}$; **Lane 4** = 0.10 $mgmL^{-1}$; **Lane 5** = 0.05 $mgmL^{-1}$; **Lane 6** = 0.025 $mgmL^{-1}$; and, **Lane 7** = 0.0125 $mgmL^{-1}$; *Lb fermentum* FM7 cells were 10 to 14-hour old.

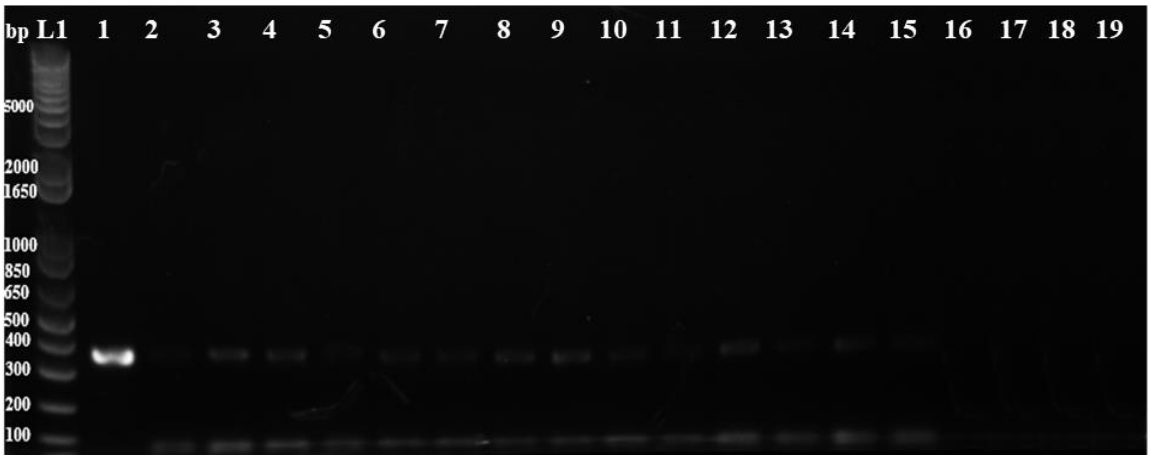


Figure 4. Amplified DNA bands of MNP-F1-captured *Lb. fermentum* FM7 cells through PCR from spiked AFM with 20% molasses in varying MNP-F1 concentrations with 10^4 $CFUmL^{-1}$ cells (Lanes 2 to 7) **and different $CFUmL^{-1}$ of *Lb. fermentum* FM7 cells on 1% Agarose gel electrophoresis.** Stained with GelRed™ run under 100V for 40 mins. Target product size of *Lb. fermentum* is 337 bp. L = 1µL 1Kb Plus DNA Ladder (Invitrogen™); Lanes 1 to 7 were loaded with 5 µL from 50 µL 10^4 $CFUmL^{-1}$ cells + 400 µL AFM (20% molasses) with 50 µL of varying MNP-F1 concentrations in: **Lane 1** = gDNA of *Lb. fermentum* FM7 (by CTAB extraction), **Lane 2** = 0.5 $mgmL^{-1}$, **Lane 3** = 0.25 $mgmL^{-1}$, **Lane 4** = 0.10 $mgmL^{-1}$, **Lane 5** = 0.05 $mgmL^{-1}$, **Lane 6** = 0.025 $mgmL^{-1}$, and **Lane 7** = 0.0125 $mgmL^{-1}$; and, Lanes 8 to 14 were loaded with 5µL from 50µL MNP-F1 (0.0125 $mgmL^{-1}$) + 400µL AFM (20% molasses) + 50µL of different $CFUmL^{-1}$ of *Lb. fermentum* FM7 cells in: **Lane 8** = 10^7 $CFUmL^{-1}$, **Lane 9** = 10^6 $CFUmL^{-1}$, **Lane 10** = 10^5 $CFUmL^{-1}$, **Lane 11** = 10^4 $CFUmL^{-1}$, **Lane 12** = 10^3 $CFUmL^{-1}$, **Lane 13** = 10^2 $CFUmL^{-1}$, and **Lane 14** = 10^1 $CFUmL^{-1}$; Control 1: NO MNPs: **Lane 15** = 50µL of 10^4 $CFUmL^{-1}$ + 450µL of 0.85% NaCl; **Lane 16** = 50µL of 10^3 $CFUmL^{-1}$ + 450µL of 0.85% NaCl; **Lane 17** = 50µL of 10^2 $CFUmL^{-1}$ + 450µL of 0.85% NaCl; **Lane 18** = 50µL of 10^1 $CFUmL^{-1}$ + 450µL of 0.85% NaCl; and, Control 2: NO CELLS: **Lane 19** = 50µL of MNP-F1 (0.0125 $mgmL^{-1}$) + 450µL of 0.85% NaCl. The 10 to 14-hour old *Lb. fermentum* FM7 cells were added during 10^{th} hour of the fermentation run. AFM contained *S. cerevisiae* BIOTECH 2030 and 20% molasses.