cancers

MDPI

*Supplementary Materials*

# Live Biotherapeutic *Lactococcus lactis* GEN3013 Enhances Antitumor Efficacy of Cancer Treatment via Modulation of Cancer Progression and Immune System

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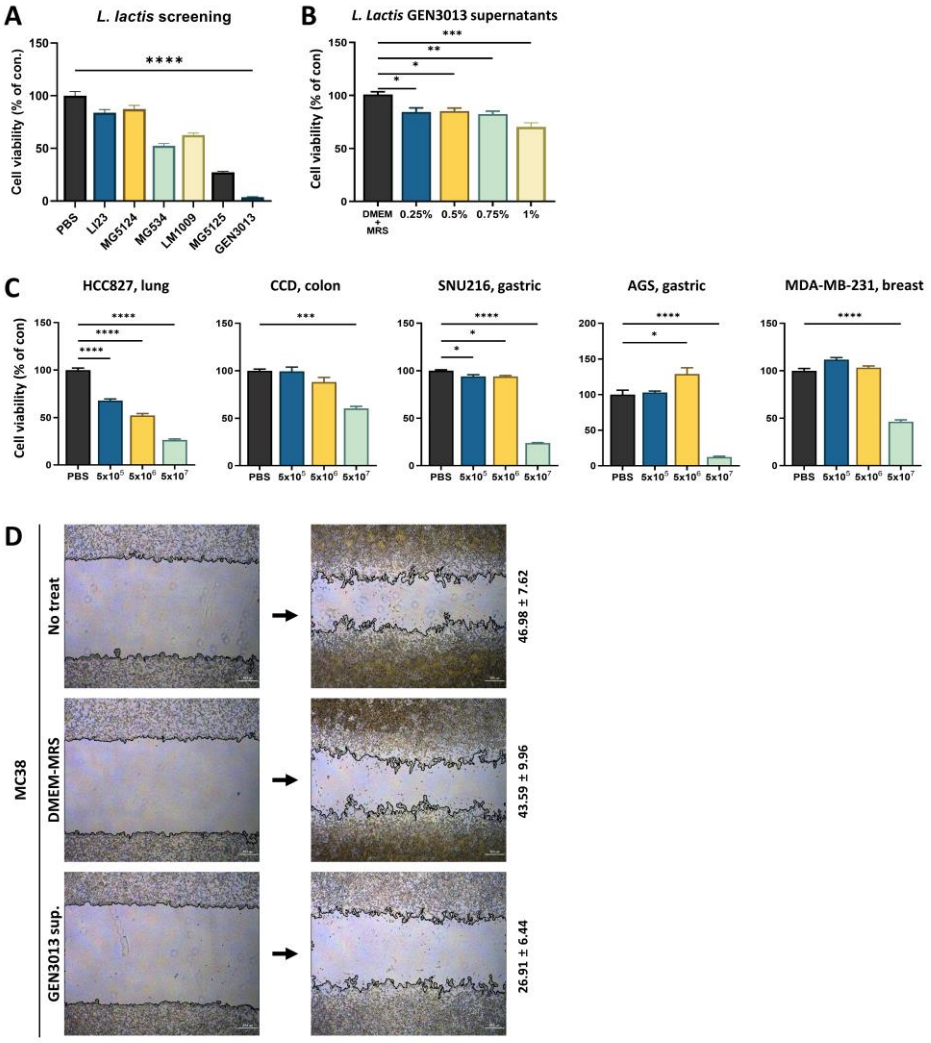
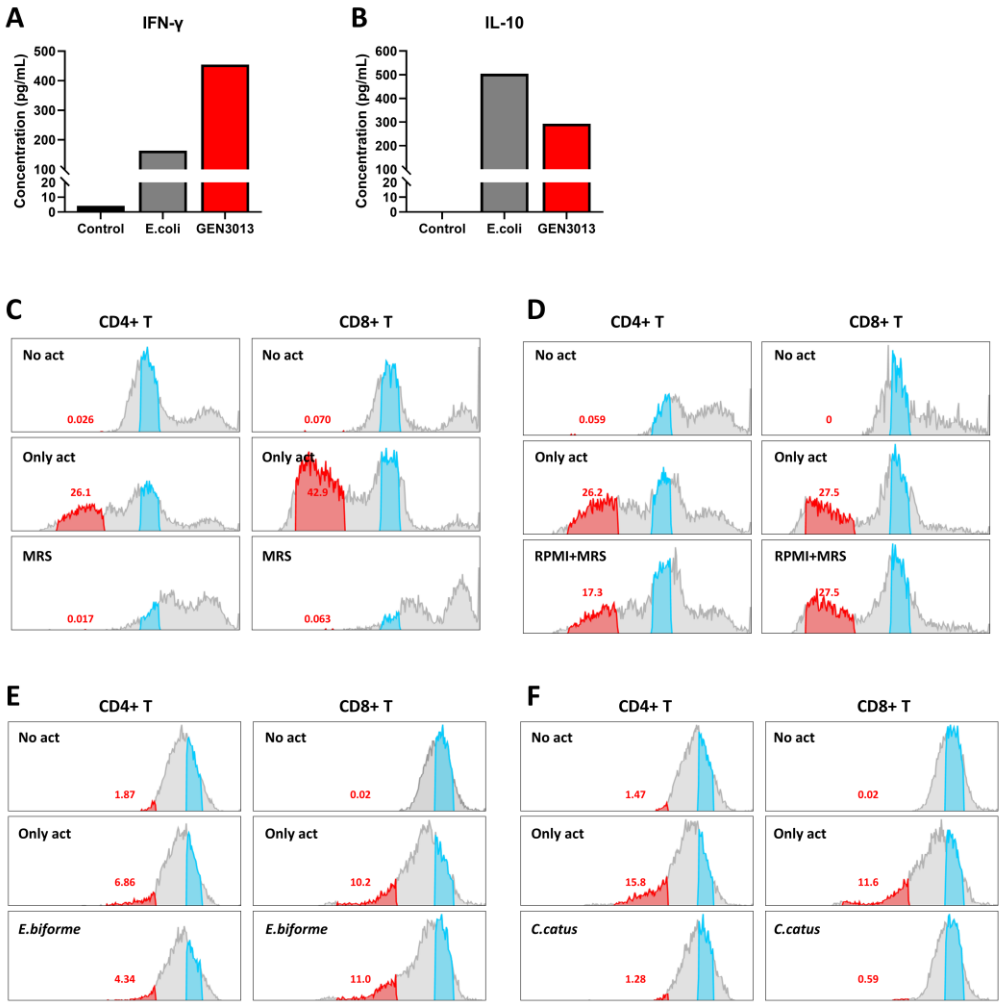



**Supplementary Figure S1. Direct effects of *Lactococcus lactis* GEN3013 on cancer cell lines.** (A) The lysates of several *Lactococcus lactis* strains were treated on MC38 cancer cell lines. The cell viability was measured by MTT assay and calculated relative to PBS control. (B) Various percent concentrations of *L. lactis* GEN3013 supernatants were treated on MC38 cells dose-dependently and incubated for 72 h. The cell viability was measured by WST-8 assay and calculated relative to MRS+DMEM control. (C) Various human cancer cell lines were treated with 0.5% *L. lactis* GEN3013 lysates in dose dependent manner and incubated for 72 h. The cell viability was measured by MTT assay and calculated relative to PBS control. (D) When treating *L. lactis* GEN3013 supernatants (26.91% ± 6.44%) to MC38 cancer cells, it also decreased the mobility of the cancer cells compared to DMEM-MRS mixture control (43.59% ± 9.96%) or no treat control (46.98% ± 7.62%).




**Supplementary Figure S2. Immune-boosting effects of *Lactococcus lactis* GEN3013**. Human T cell activation was observed by ELISA of IFN-gamma (A) and IL-10 (B) with or without *L. lactis* GEN3013 treatment. Preliminary T cell assay was conducted with two controls, (C) MRS media (bacteria culture media) and (D) a mixture of MRS and RPMI (the culture medium for T cells). Two additional species, which are known not to harm humans, were confirmed by T cell proliferation assay. (E) *E. biforme* had no significant effect T cell proliferation and (F) *C. catus* inhibited the T cell proliferation.

**Supplementary Table S1. Conditions for metabolic analysis of *Lactococcus lactis* GEN3013-derived metabolites**

| | Molecular Weight | Theoretical m/z | Adducts | Retention Time (min) | GEN3013 (1) | GEN3013 (2) | GEN3013 (3) | GEN3013 (4) | GEN3013 (5) |
|---|---|---|---|---|---|---|---|---|---|
| Vaccenic acid | 282.256 | 283.2631568 | [M+H]+ | 30.260 | 6.886 | 6.753 | 6.825 | 6.833 | 6.874 |
| S-Adenosylmethionine | 398.137 | 399.1445153 | [M+H]+ | 0.810 | 6.927 | 6.636 | 6.799 | 6.699 | 6.733 |
| Oleic acid | 282.256 | 299.2591686 | [M-H+H2O]- | 30.230 | 5.005 | 4.908 | 4.963 | 5.040 | 5.154 |
| N,N-Dimethylformamide | 73.053 | 145.0982512 | [2M-H]- | 0.650 | 5.093 | 5.152 | 5.382 | 5.107 | 5.298 |
| Methylthioadenosine | 297.090 | 298.0968368 | [M+H]+ | 5.950 | 7.371 | 7.235 | 7.351 | 7.320 | 7.249 |
| Indole lactic acid | 205.074 | 188.070605 | [M+H-H2O]+ | 1.660 | 7.781 | 7.289 | 7.428 | 7.331 | 7.382 |
| GABA (gamma-aminobutyric acid) | 103.063 | 102.0560521 | [M-H]- | 0.680 | 5.661 | 5.394 | 5.418 | 5.239 | 5.415 |
| Citrate | 192.027 | 191.0197261 | [M-H]- | 0.600 | 6.747 | 6.296 | 6.329 | 6.327 | 6.518 |
| Betaine | 117.079 | 116.0717021 | [M-H]- | 0.700 | 6.193 | 5.666 | 5.770 | 5.610 | 5.772 |

Normalized log10, 5 technical replicates