Biogenic Selenium Nanoparticles Produced by the Probiotic *L. casei* and Selenium Nanoparticles-enriched *L. casei* as Bioactive Dietary Compounds Against Colon Cancer

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Background

Selenium nanoparticles against colon cancer

Selenium has been reported to be effective in cancer prevention/therapy [1,2], to inhibit metastasis and to enhance the therapeutic effects of conventional anticancer therapeutic schemes [3]. Recent clinical trials have demonstrated the anticancer properties of selenium against colorectal cancer in specific [4]. There is though, a very narrow window between the beneficial/therapeutic dose of selenium and the dose that exerts toxicity [5]. Se nanoparticles (SeNps), possess distinct physicochemical characteristics compared to other selenium forms that render them less toxic and more bioavailable [6].

Rationale of using biogenic selenium nanoparticles by *L. casei*

Today, the most commonly used method for the synthesis of SeNps is chemical reduction. Various microorganisms though have been reported to synthesize SeNps through their detoxification mechanisms [7] or redox homeostasis [8]. These biogenic SeNps are characterized by such variety and specificity that cannot be achieved by the available physicochemical synthesis methods [9]. Among the various microorganisms that are able to produce SeNps is the probiotic Lactobacillus casei ATCC 393 (LC) [10] which our team has previously shown that exerts antitumor and immunomodulatory effects against colon cancer [11, 12].

Aim

To use LC for the synthesis of biogenic SeNps and to evaluate the potential anticancer activity of LC-derived SeNps (SeNps) and SeNps-enriched LC (LCSe) as bioactive compounds administered orally against colon cancer.

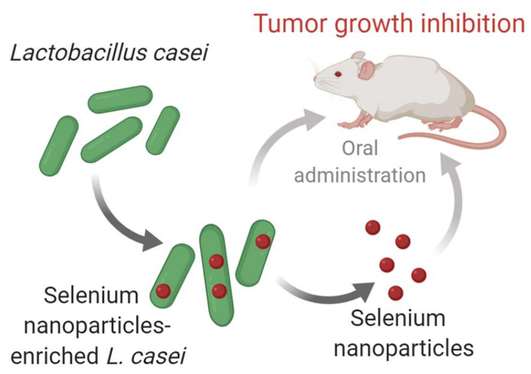

Fig 1. Outline of the study.

Materials & Methods

- Probiotic bacterial strain *L. casei* ATCC 393 cultured in the presence of $NaHSeO_3$ as a Se source.
- Extraction of SeNps with ultrasonication, NaOH treatment and vacuum filtration.
- UV-Vis, SEM-EDS, XRD analyses for SeNps characterization.
- Murine CT26 and human HT29 colon cancer cell lines.
- SRB assay for cell growth analysis.
- Syngeneic CT26 transplantable BALB/c mouse tumor model: 24 male mice.
- ELISA for the serum cytokine analysis.
- Proteome Profiler Human Apoptosis Antibody Array kit for the protein expression levels detection
- Flow cytometry and fluorescence confocal microscopy for the protein cellular localization analysis.

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Results and Discussion

LC synthesizes intracellularly spherical SeNps of 360 nm

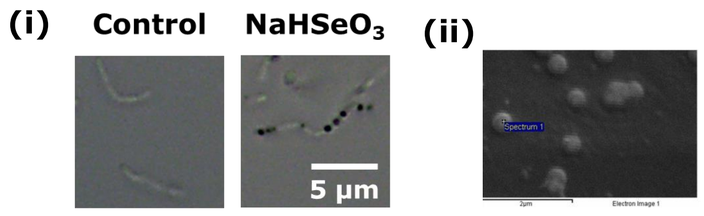


Fig 2. (i) Brightfield images of the bacteria. (ii) SEM images of extracted SeNps.

1,500 μg of SeNps were purified from 10^{10} CFUs of bacteria.

Cancer specific antiproliferative activity of SeNps and LCSe *in vitro*

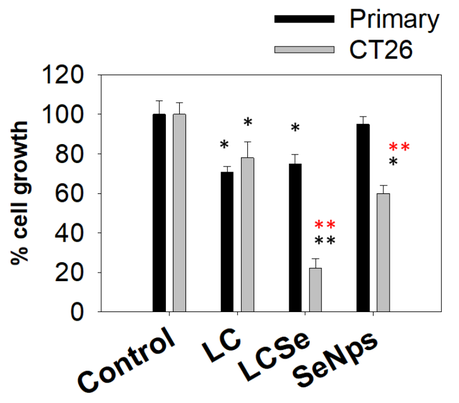


Fig 3. Treatment with 10^8 CFUs/ml of LC, LCSe or 15 μg/ml SeNps for 24 h.

**Treated vs control.*
**Cancer (CT26) vs healthy (Primary).*

Significant inhibition of tumor growth in mice receiving SeNps or LCSe *per os*

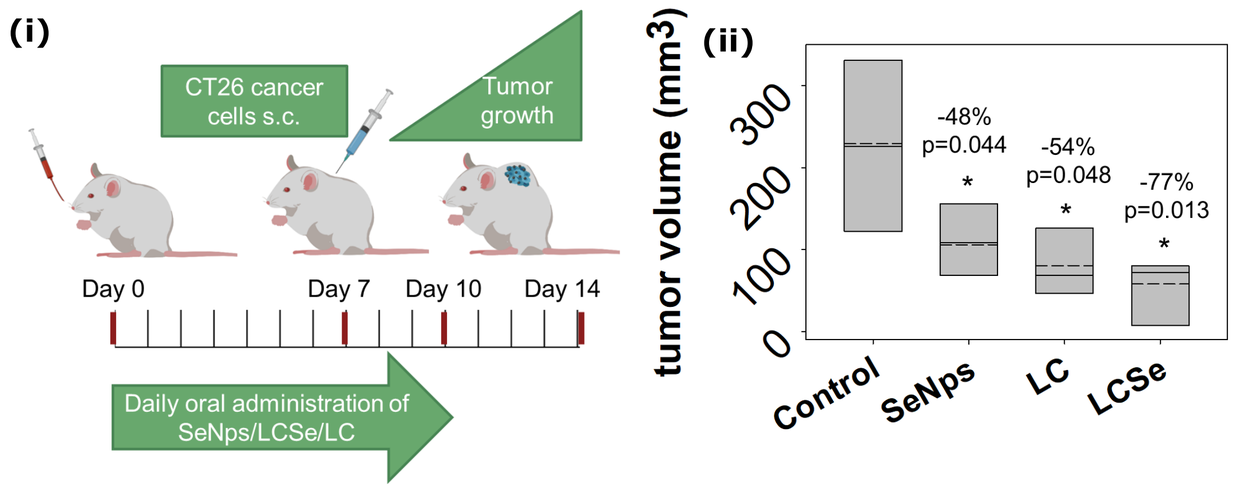
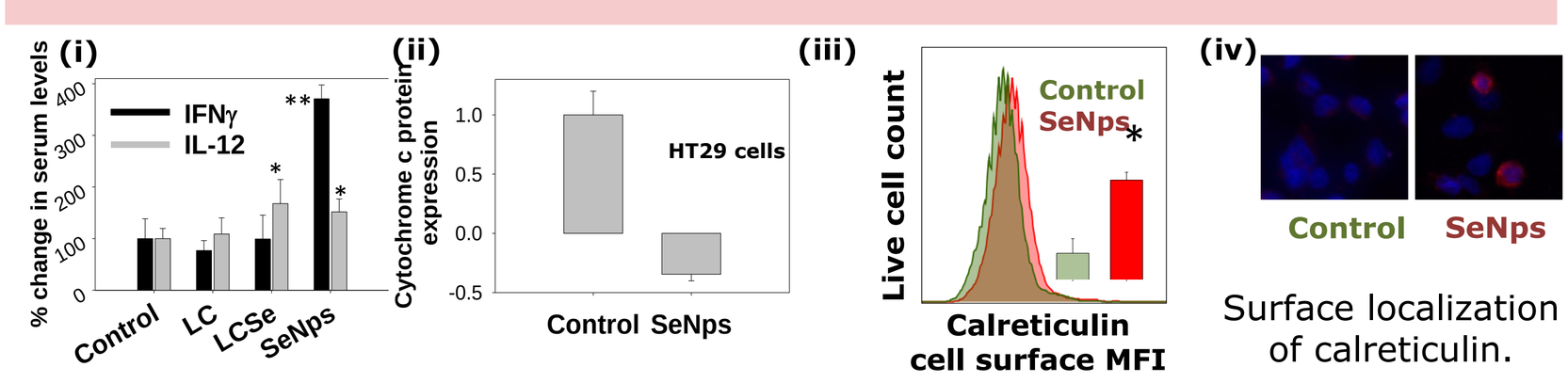


Fig 4. (i) Mice received 10^9 CFUs of LC or LCSe or 150 μg of SeNps once per day for ten days prion subcutaneous injection of cancer cells **(ii)** Tumor size.

No indication of toxicity.

SeNps exert immunostimulatory effects in colon cancer cells



Surface localization of calreticulin.

Fig 5. (i) Elevation of IFNγ and IL-12 serum levels in tumor bearing mice. **(ii)** Decreased expression of cytochrome c and **(iii-iv)** translocation to the cell surface of calreticulin in SeNps-treated HT29 cells.

Conclusions

To the best of our knowledge this is the first comparative study assaying the anticancer effects of SeNps synthesized by a microorganism, the SeNps-enriched microorganism and the sole microorganism. Our results demonstrate the strong potential of nanoparticles-enriched probiotics for cancer prevention while highlighting the exciting prospects of their exploitation in pharmaceutical and food industries. However, more investigations are needed to fully understand the underlying antitumor mechanisms.