

# Anticancer effect of probiotic fermented milk on human colon cancer cells

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## Relevance

- ✓ Colon Cancer (CC) is reported to be the third-leading cause of deaths from cancer for both males and females globally (Xi and Xu, 2021). Against CC, anticancer therapies are used with varying degrees of success. These therapies are costly and have adverse effects.
- ✓ Due to these issues, researchers are becoming more interested in adopting natural foods as well as dairy products (including fermented milks) that contain anticancer components as a preventive as well as management option for colon cancer.
- ✓ Fermentation of milk by lactic acid bacteria elaborate a range of metabolites possessing health benefits. Such metabolites may include organic acids, short-chain fatty acids, bioactive peptides and various cell surface components, among other metabolites. These metabolites have been suggested to have the potential to prevent colon cancer through a number of mechanisms such as antioxidant activity, binding and degradation of potential carcinogens, production of anticancer compounds, apoptosis induction, stimulation of the host's immune response and effects on host physiology (Chen et al, 2012, Chong, 2014, Eslami et al, 2019).
- ✓ But the anti-cancer effects are said to be specific to the strain and its metabolites. Therefore, the selection of suitable probiotic strains and their fermentation metabolites for their anticancer potential against colon cancer is essential.

## Objective

To evaluate the anticancer effect of probiotic fermented milk on human colon cancer cells.

## Methodology

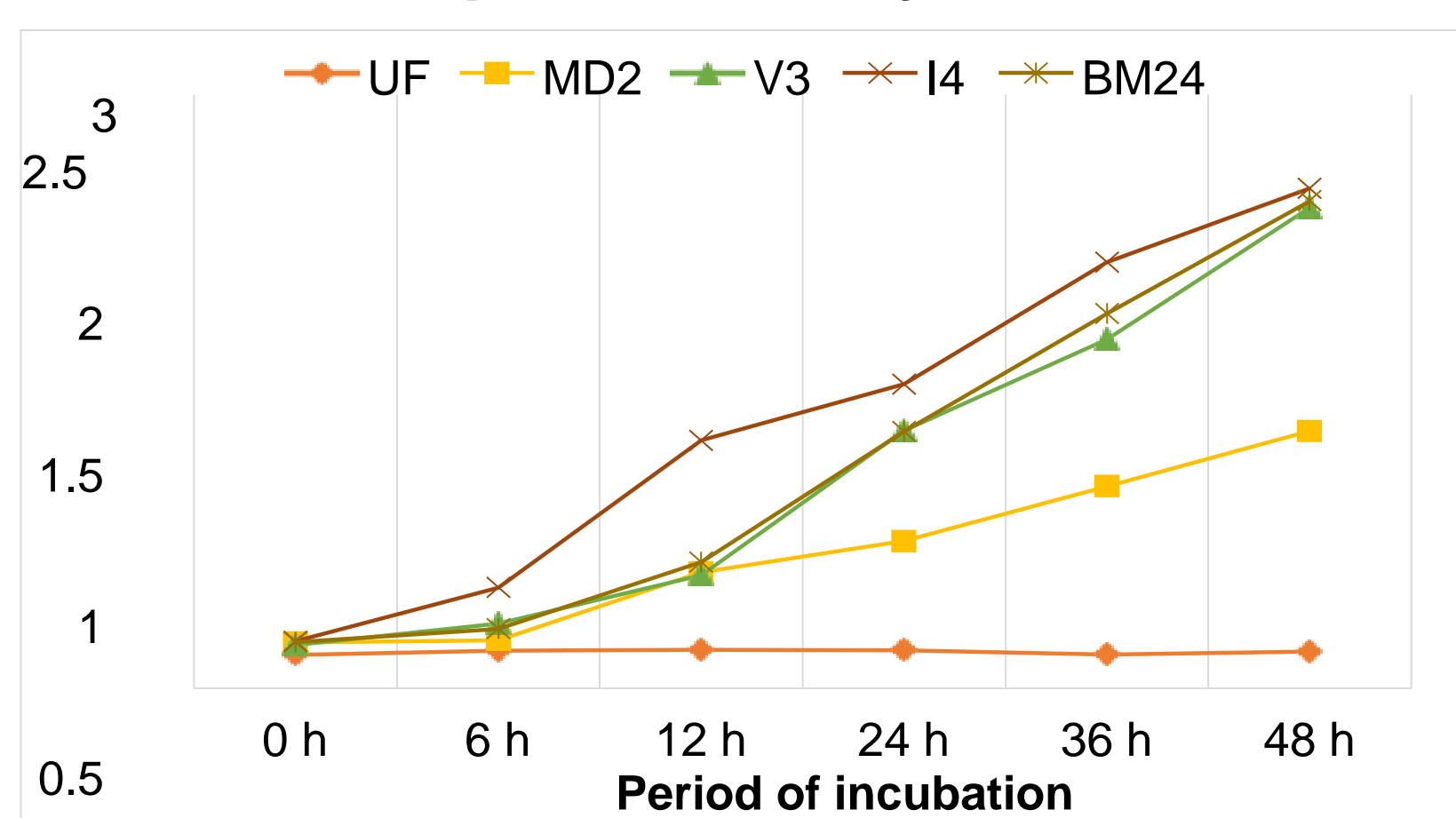
- ✓ **Screening of strains:** Reconstituted skim milk (RSM) was fermented using the strains *Streptococcus thermophilus* MTCC 5460 (MD2), *Lactobacillus helveticus* MTCC 5463 (V3), *Lactobacillus rhamnosus* MTCC 5462 (I4) and *Limosilactobacillus fermentum* (BM24). The supernatants of the fermented milks were prepared by centrifugation (14,000 × g at 4°C for 30 min.) and filtration (through a 0.22-µm membrane filter). The supernatants were screened based on the antioxidant activity (Re et al., 1999), antimicrobial activity (Delgado et al., 2001), proteolytic activity (Donkor et al., 2005), anticarcinogenic activity by nitrosamine degradation assay.
- ✓ Supernatant of most promising strain (MTCC 5463) was evaluated for antidiabetic potential in terms of α-amylase inhibitory and α-glucosidase inhibitory activity as well as for ACE-inhibitory activity (Chaudhary and Mudgal, 2020). Further, the effect of different doses of Freeze-Dried Supernatant (FDS) was evaluated on human colon cancer cell line HT-29 using MTT assay (Sevda et al., 2015). Unfermented milk (UF), pH adjusted UF (pH 4.6 using acid) and the drug 5-fluorouracil (5-FU) was used as controls.
- ✓ Gene expression study using rt-PCR (Ardestani et al., 2019) was carried out to understand the mode of action. The SYBER Green method was used to determine the expression of pro- and anti-apoptotic (Caspase-8, Bax, Bcl2, p53 and cyclinD1) genes in the HT-29 cells treated with the FDS of fermented milk for 24 h. The GAPDH gene was used as an internal control.

### Primers used in the study

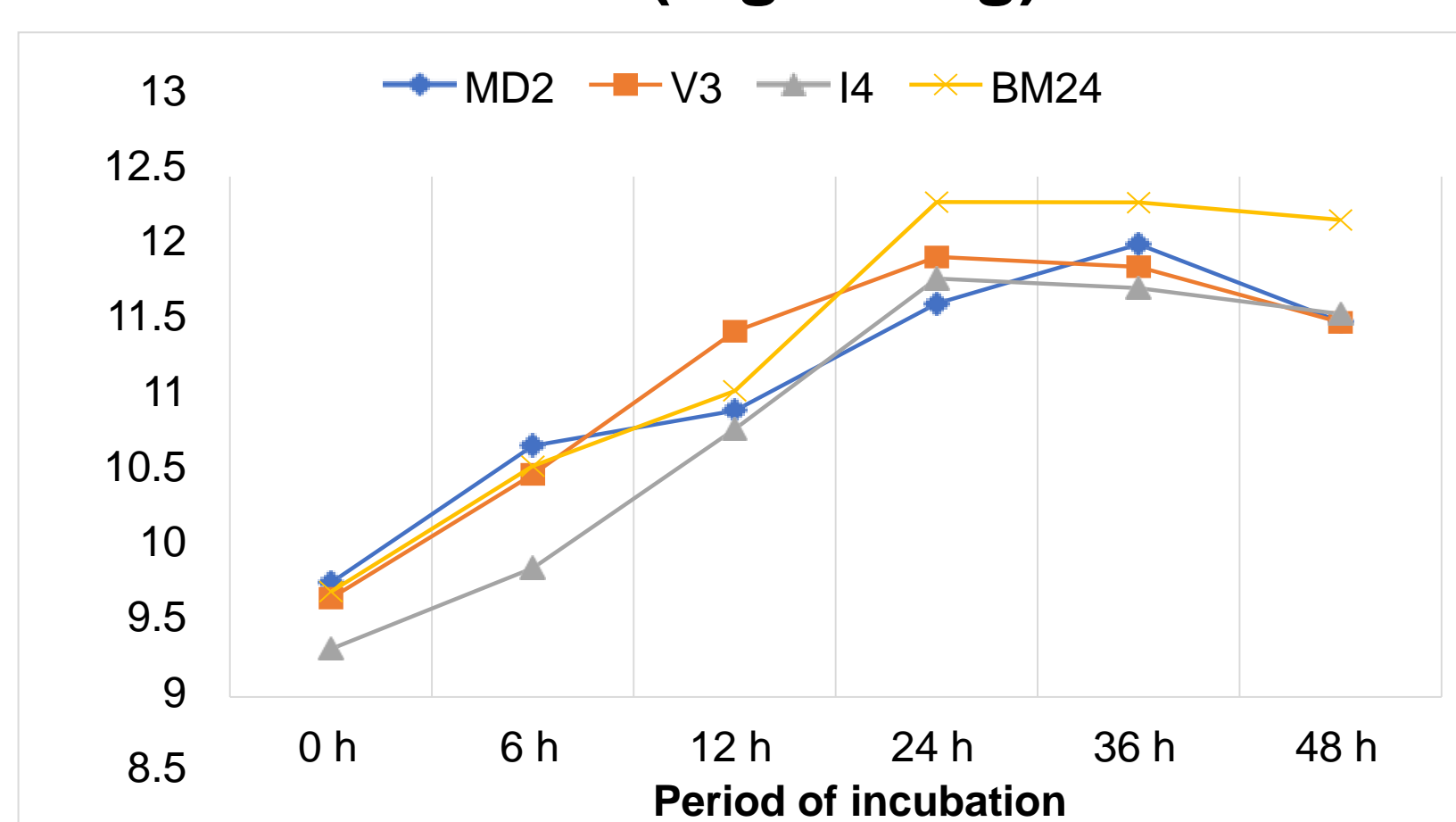
Gene	Forward	Reverse
caspase 8	GACAGAGCTTCTTCGAGACAC	GCTCGGGCATAACAGGCAAAT
Bax	CCCAGAGAGTCTTTTCCGAG	CCAGCCCATGATGGTCTGAT
Bcl-2	GGTGCCGGTTCAGGTACTCA	TTGTGGCCCTTCTTTGAGTTCG
P53	CATAGTGTGGTGGCCCTA	CACCTCAAAGCTGTCCGTC
cyclinD1	GCTGCGAAGTGGAAACCATC	CCTCTTTCGACACATTGAA
GAPDH	AGAAGGCTGGGGCTCATTG	AGGGCCATCCACAGTCTTC

## Results & Discussion

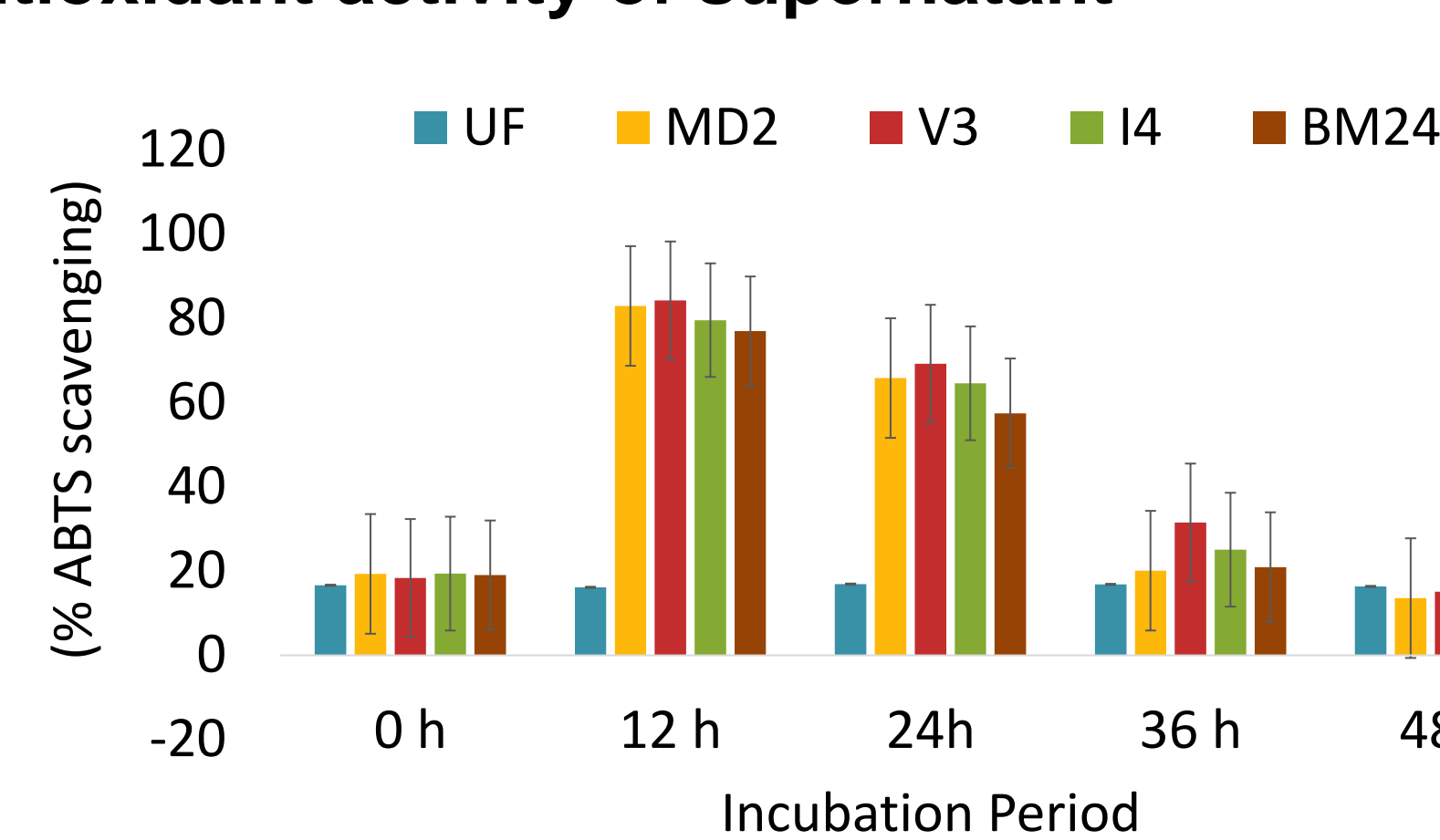
### Lactic acid production by strains in RSM



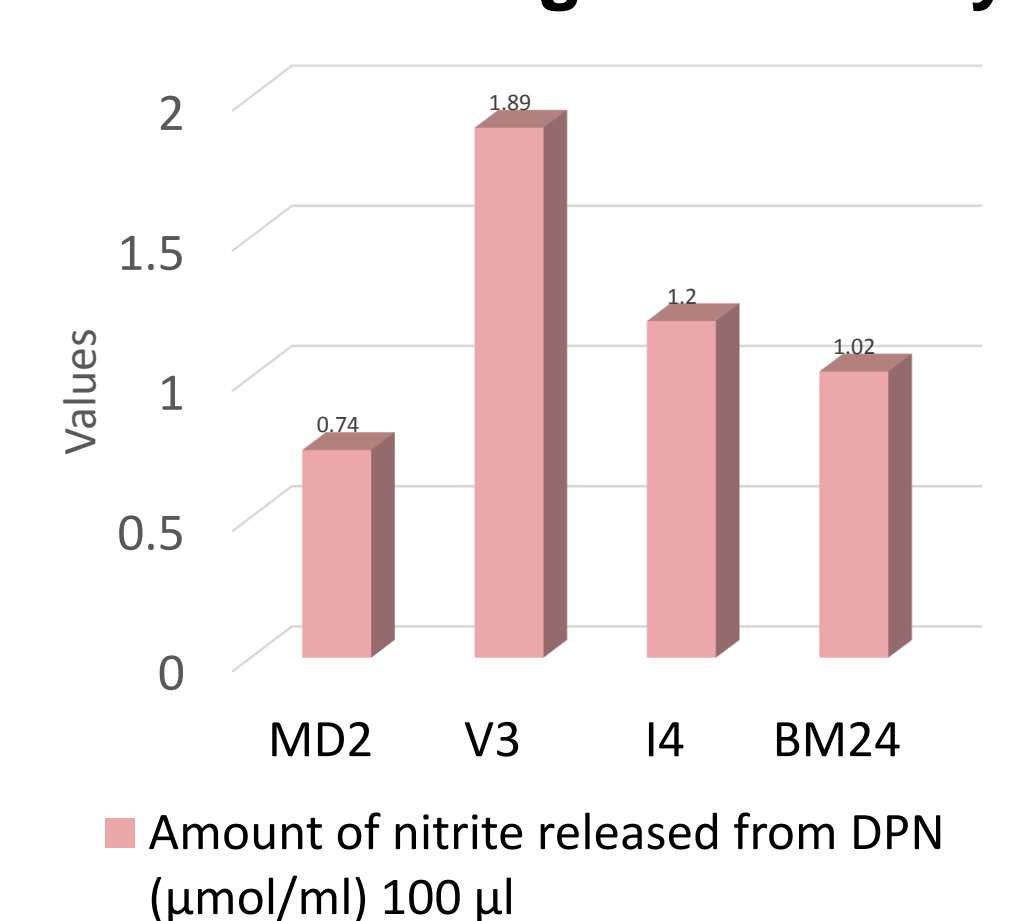
### LAB counts (log CFU/g) in RSM



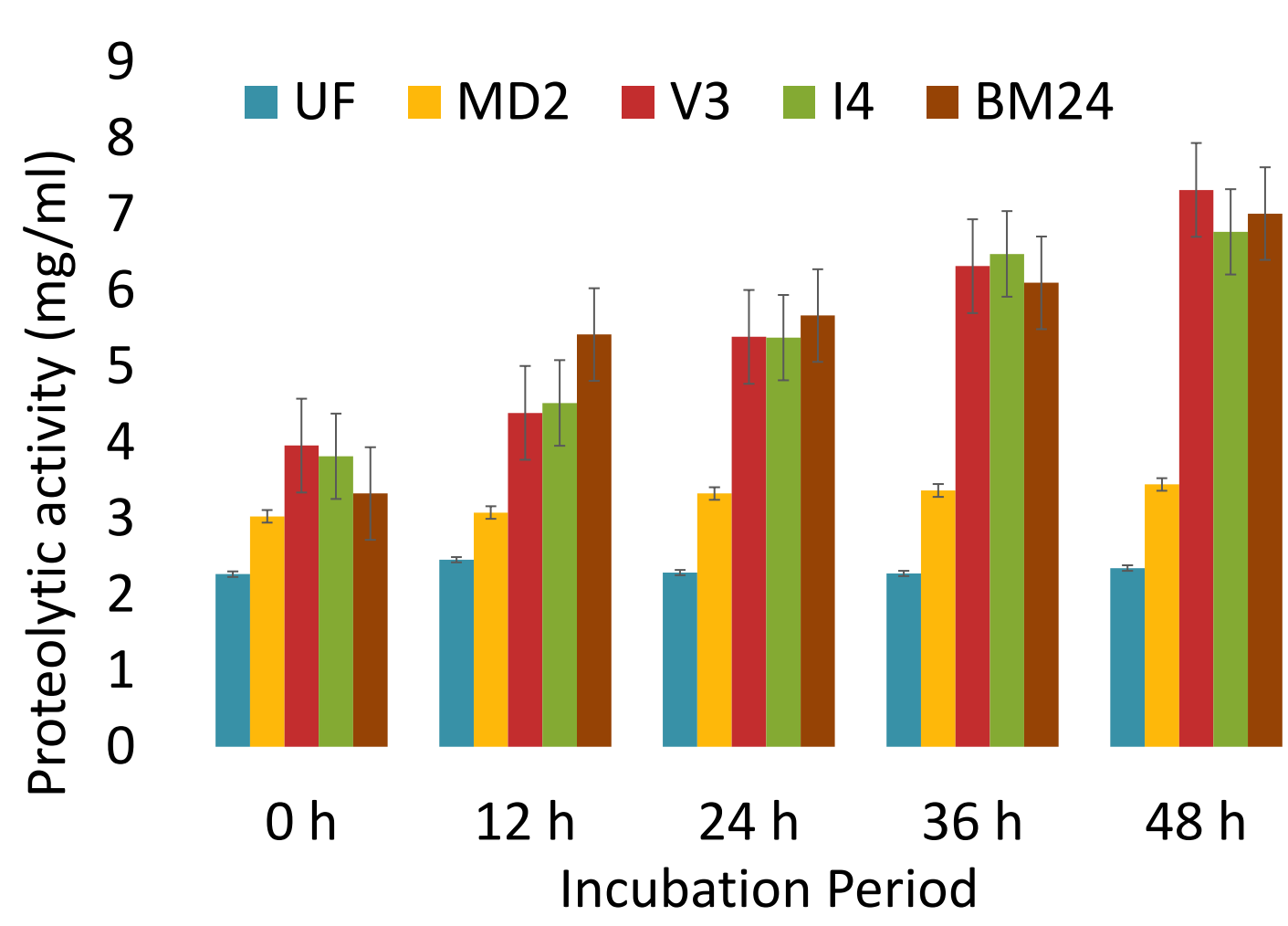
### Antioxidant activity of supernatant



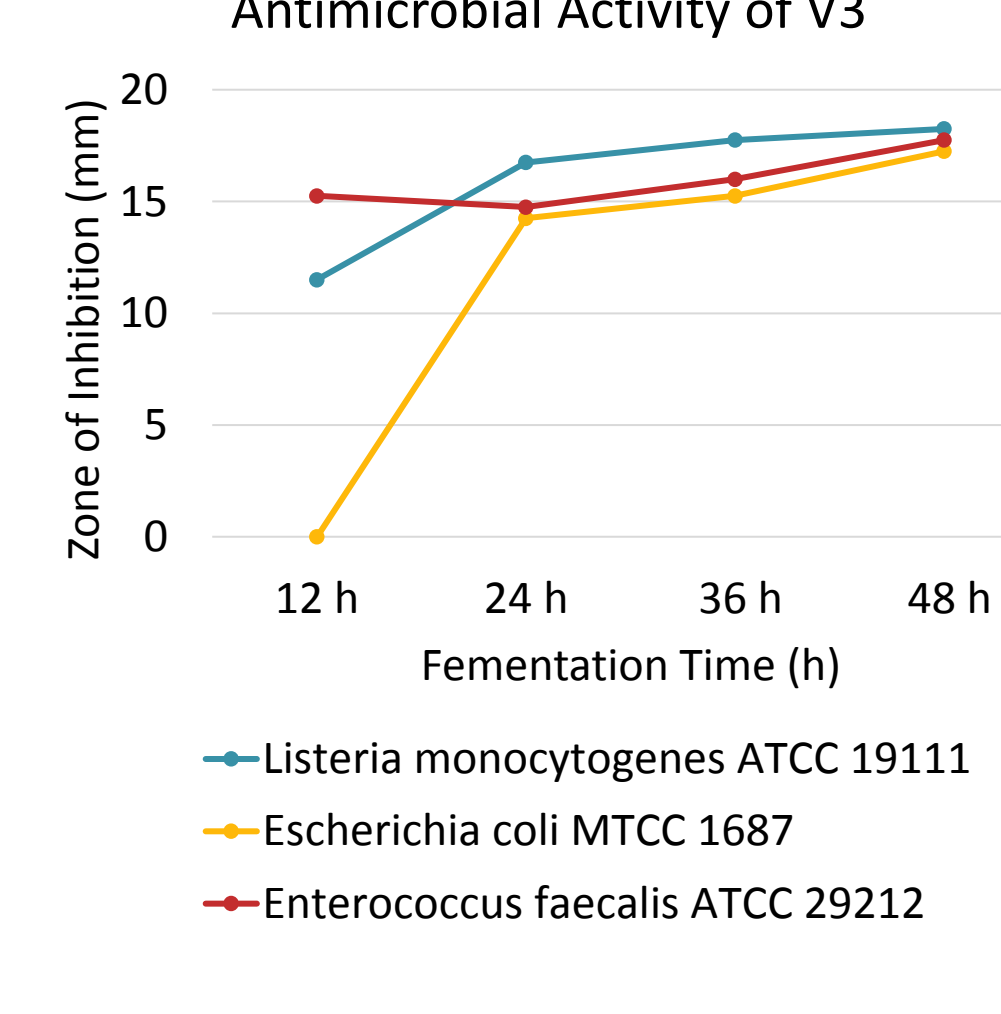
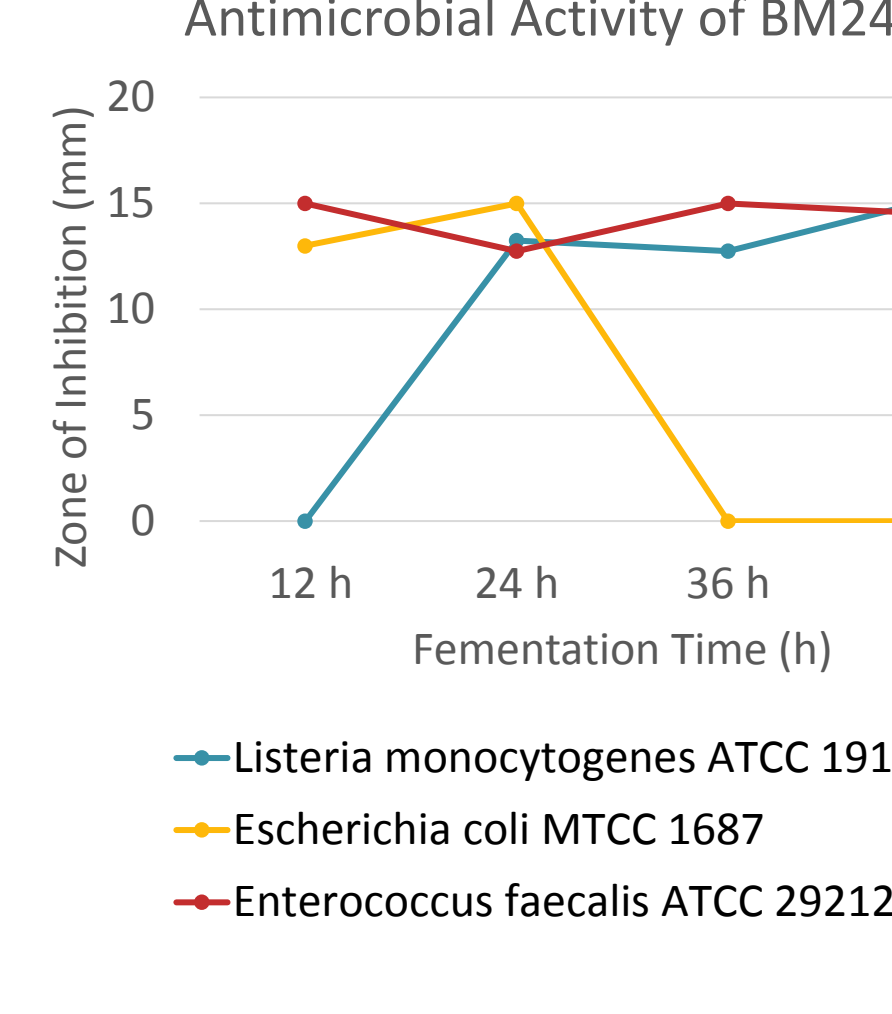
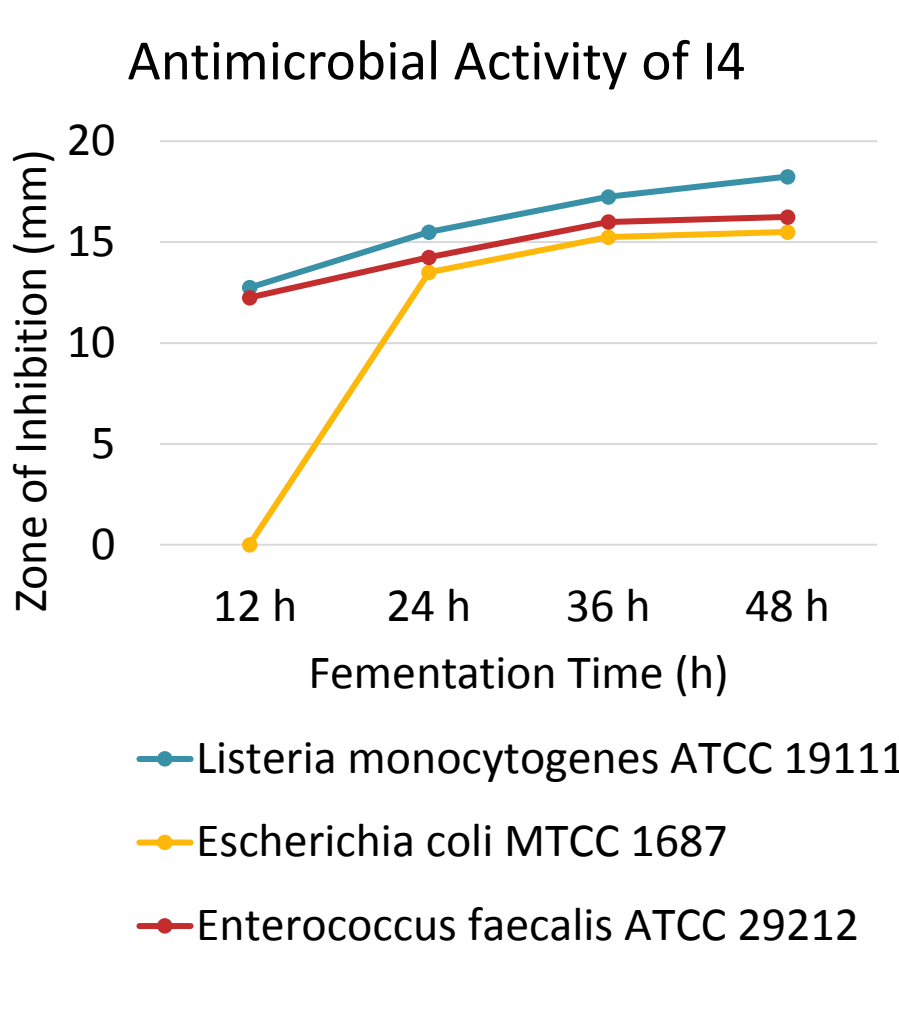
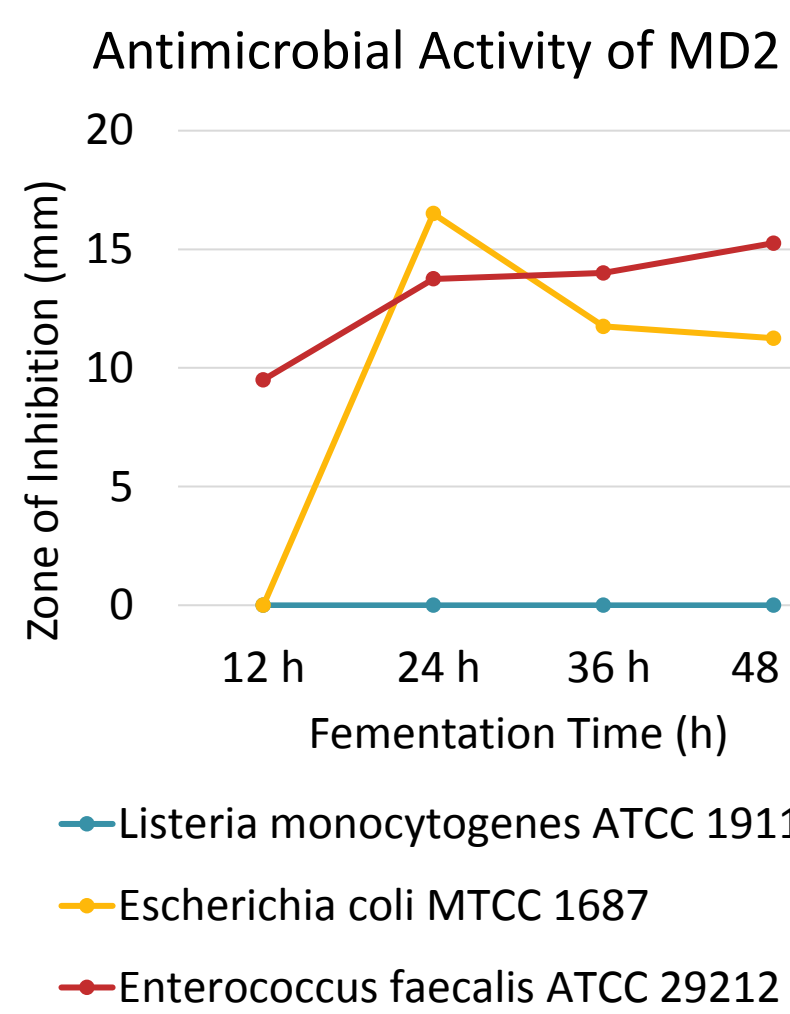
### Anti-carcinogenic activity



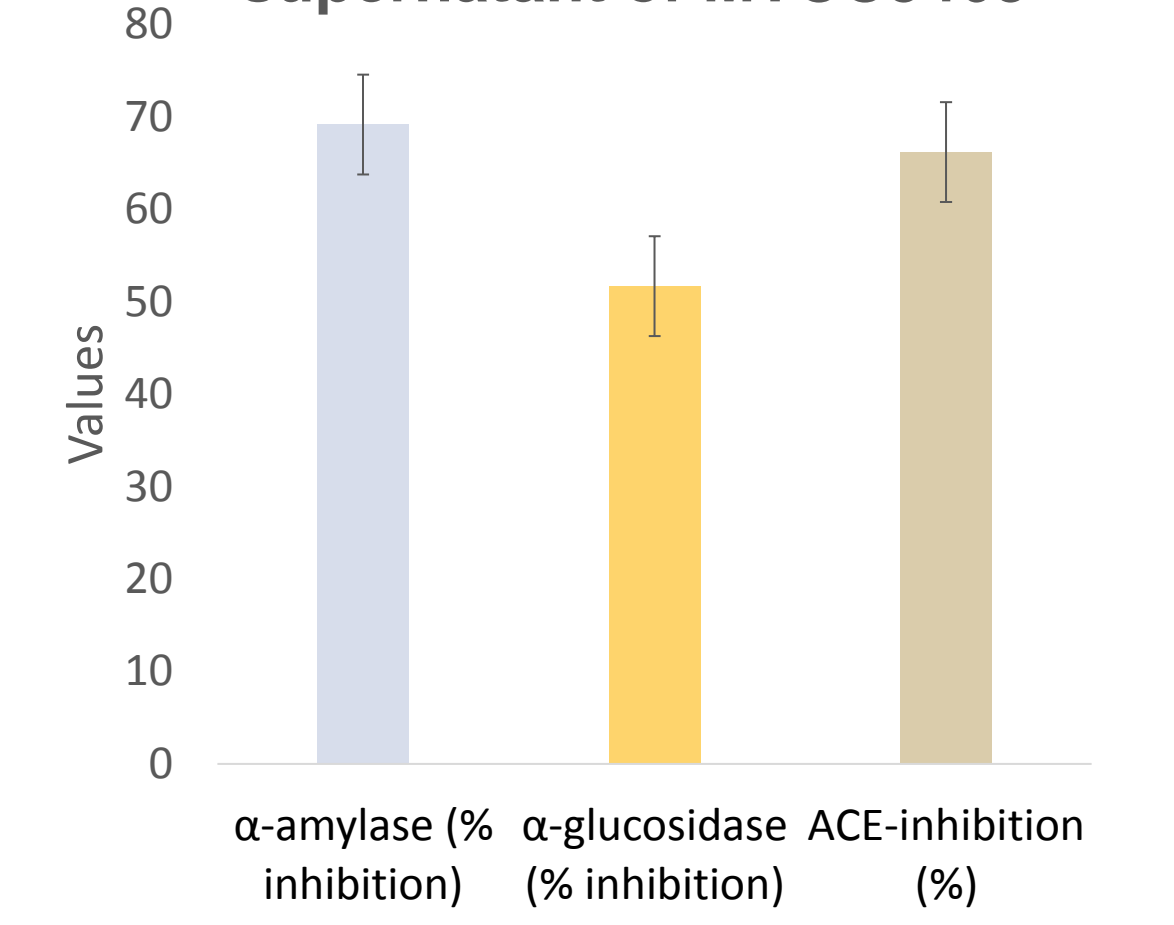
### Proteolytic activity (mg/ml) of supernatant



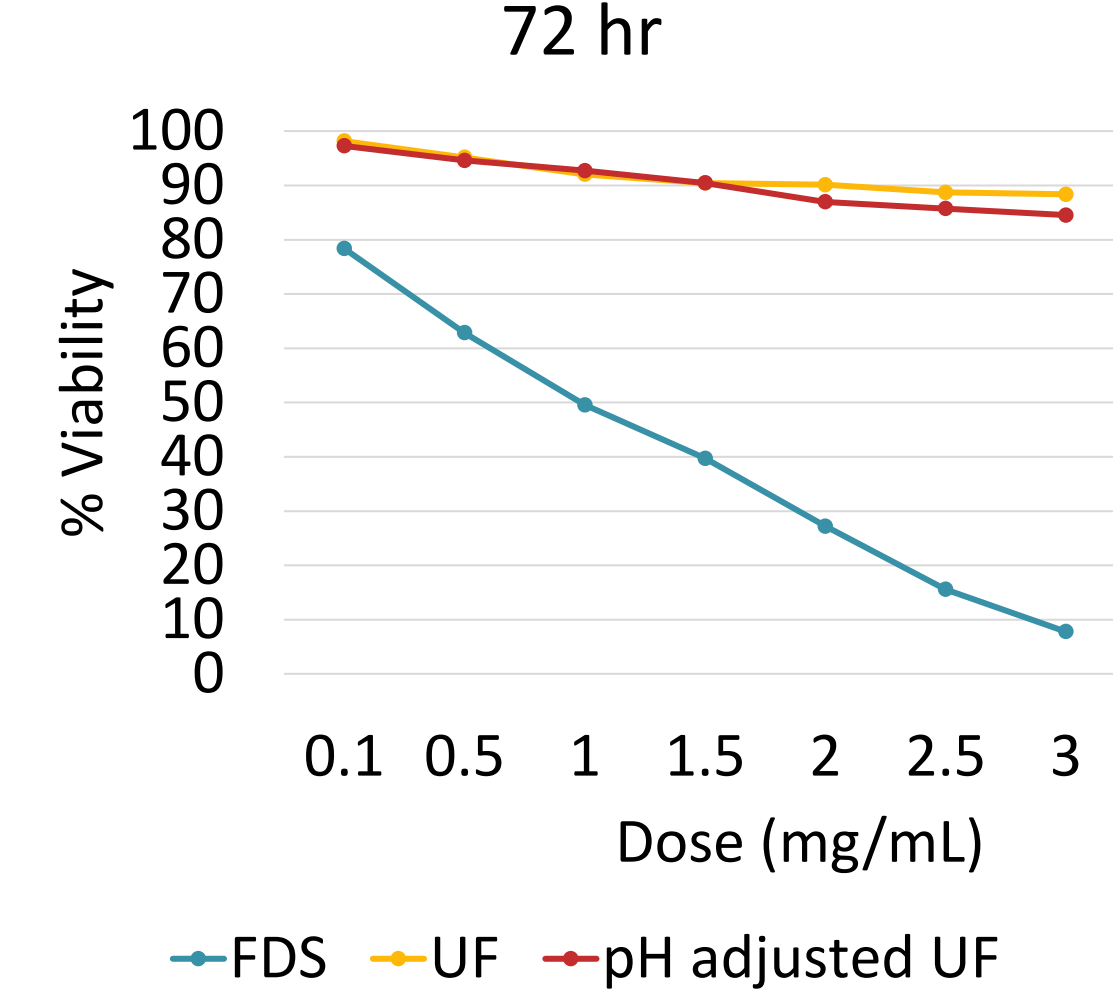
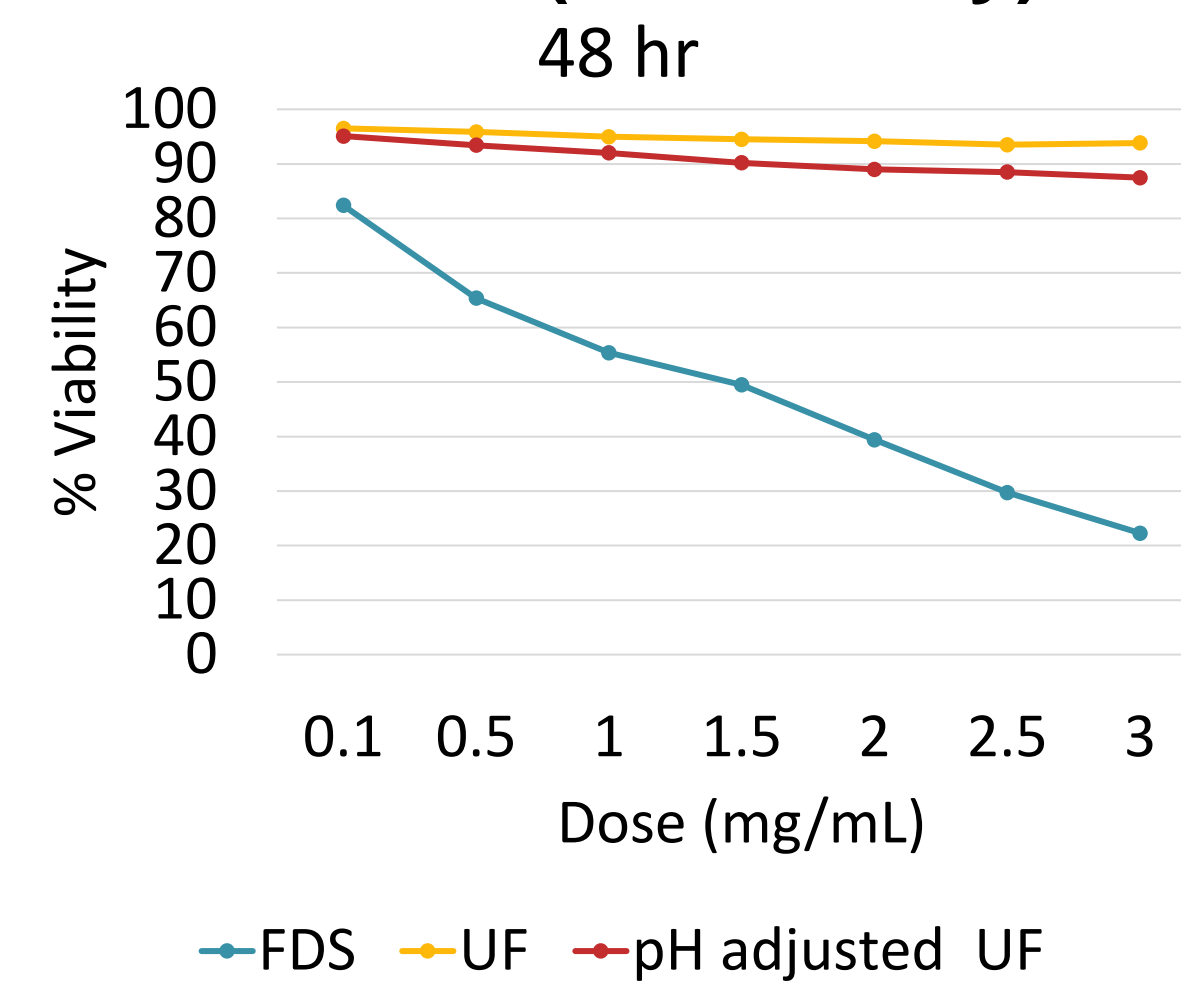
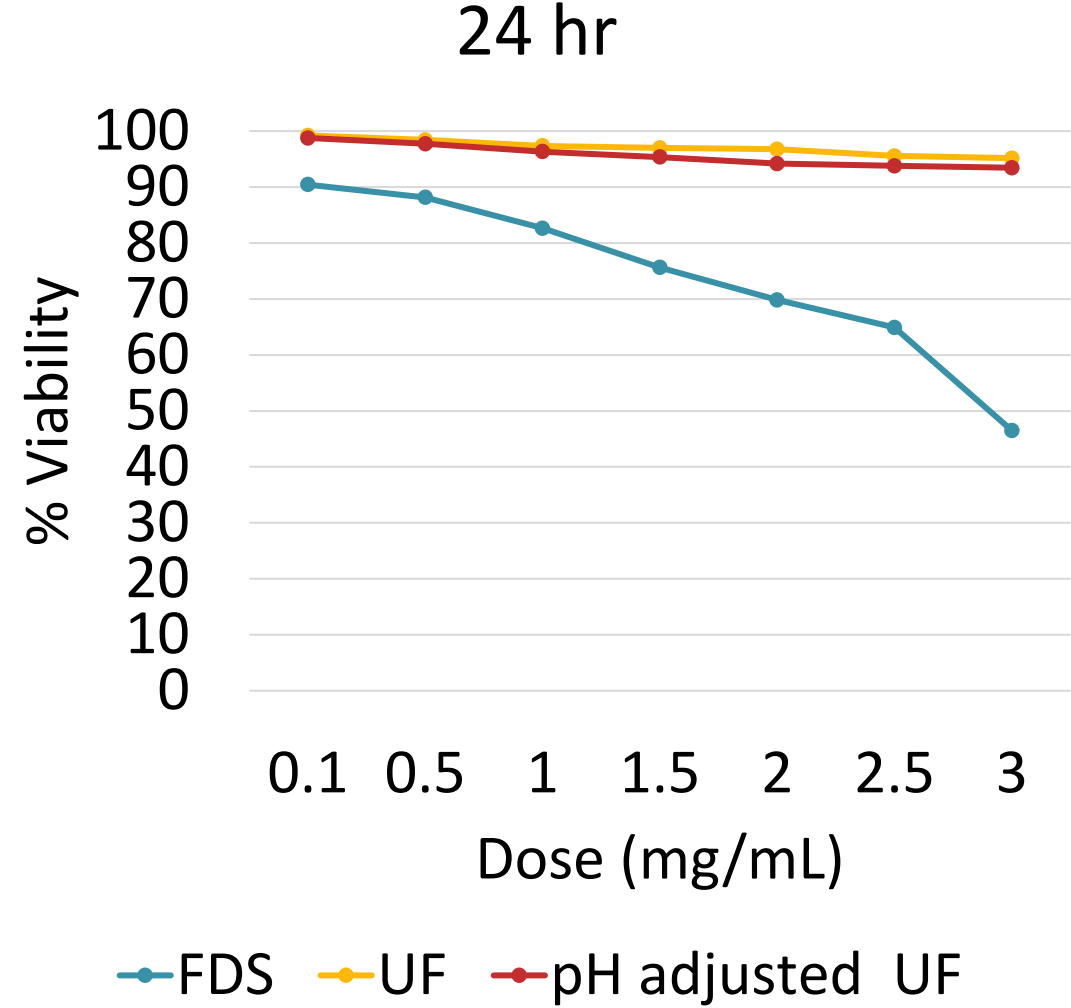
### Antimicrobial activity of supernatant



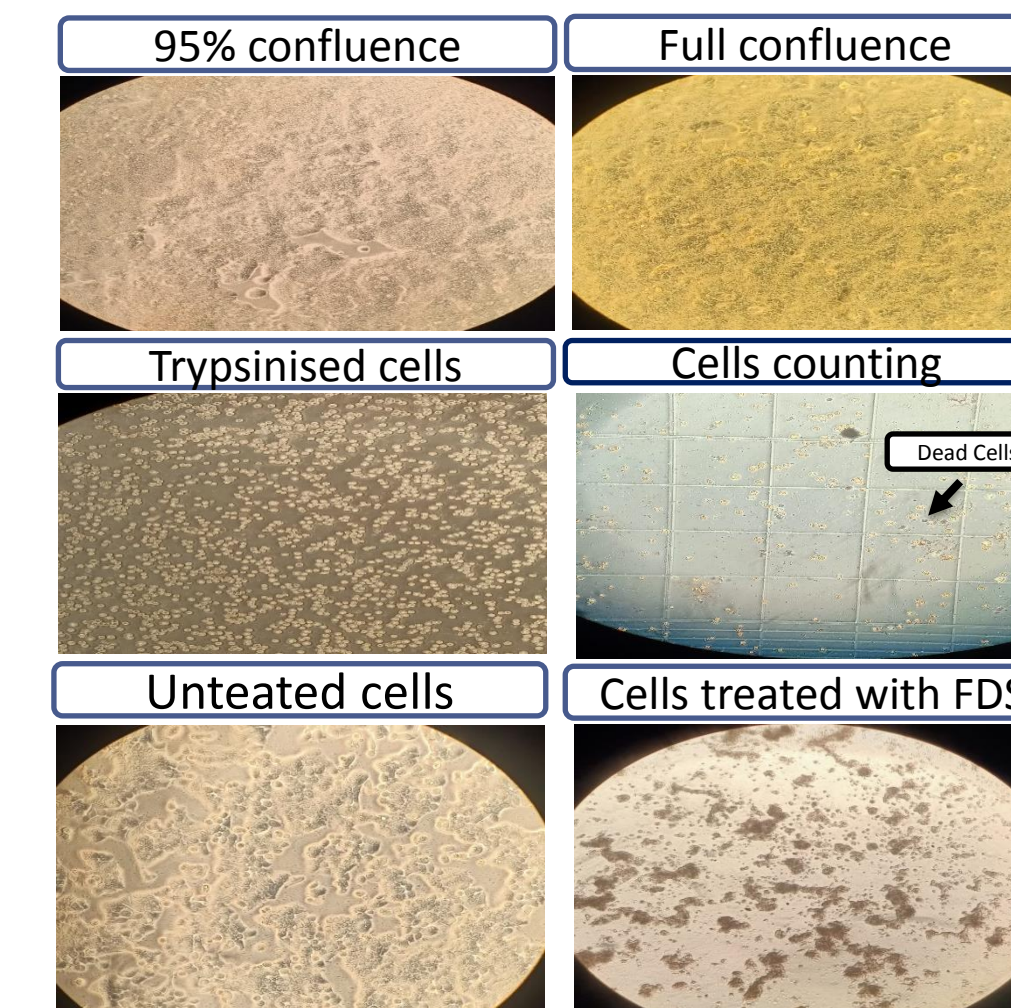
### α-Amylase Inhibition, α-Glucosidase Inhibition & ACE-Inhibition Activities of supernatant of MTCC5463



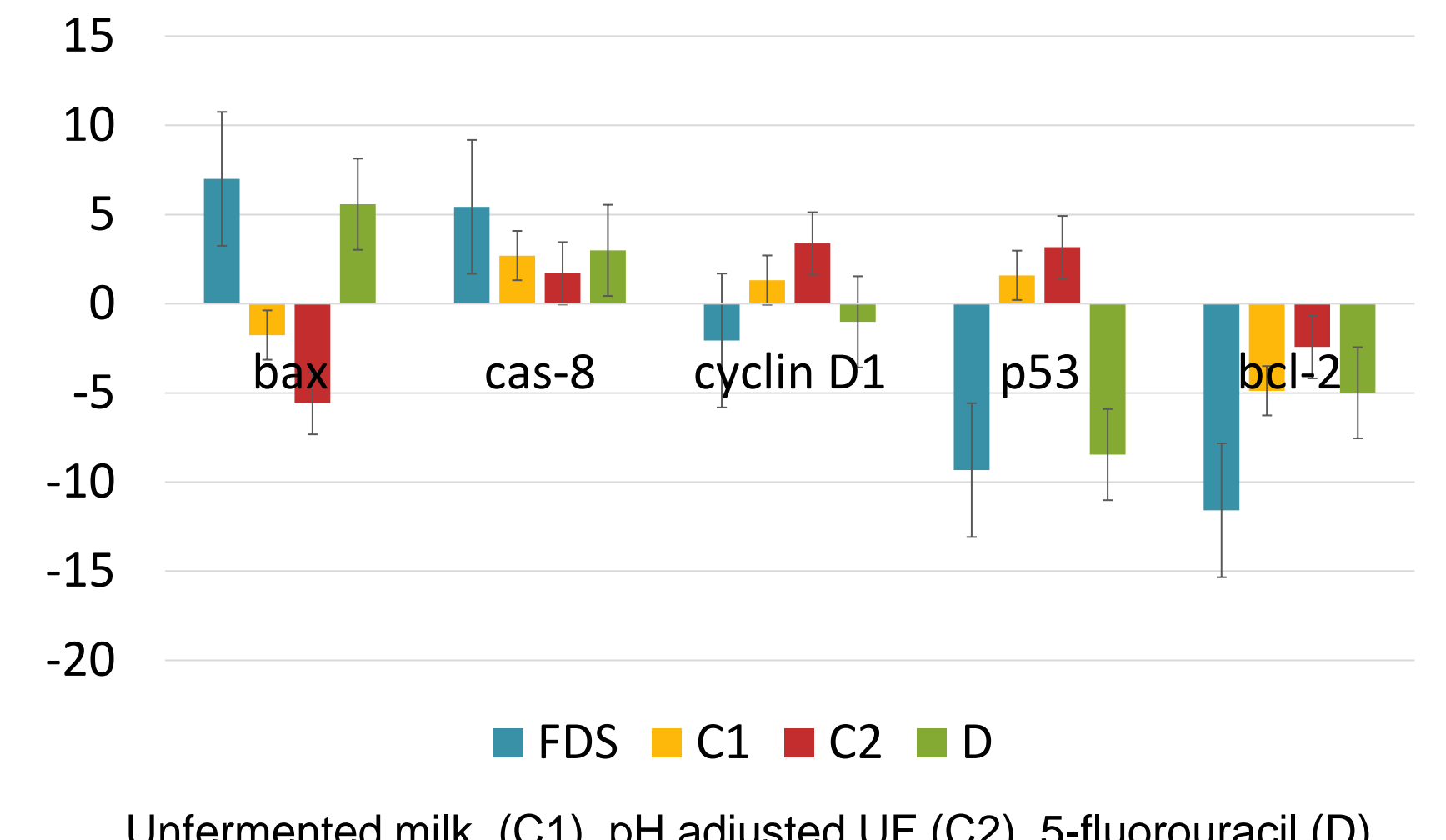
### Effect of fermented milk supernatant on viability of HT-29 cells after 24 h, 48 h and 72 h of treatment (MTT Assay)



### HT-29 cells under a light microscope (400x)



### Effect of supernatant of fermented milk on expression levels of pro- and antiapoptotic genes



## Salient Findings and Conclusion

- ✓ Supernatant of the fermented milk of *Lactobacillus helveticus* MTCC 5463 (V3) showed highest antioxidant activity, antimicrobial activity, proteolytic activity and anticarcinogenic activity compared to other strains. FDS of V3 showed promising α-amylase inhibitory activity (69.18 %), α-glucosidase inhibitory activity (51.68 %) and ACE-inhibitory activity (66.19 %).
- ✓ Fermented milk supernatant significantly decreased the growth of HT-29 compared to unfermented control. IC50 of freeze-dried supernatant against HT-29 cells were 2.9, 1.5 and 1.0 (mg/mL) at 24, 48 and 72 h, of treatment respectively. Cytotoxicity levels were dose and time dependent. RT-PCR results revealed upregulation of pro-apoptotic genes (Bax and Caspase-8) in HT-29 cells treated with supernatant of fermented milk in comparison with the untreated control cells. Anti-apoptotic genes Bcl-2, cyclin D1 and p53 were downregulated. The ability of probiotics and its metabolites to regulate cellular apoptosis may be an essential step in CRC prevention.
- ✓ The findings of the study demonstrated that fermented milk of *Lactobacillus helveticus* MTCC 5463 possess promising anticancer activity against colon cancer cell line HT-29 and the probable mode of action may be apoptosis induction through upregulation and down regulation of genes involved in apoptosis, which may be beneficial in the prevention and management of colon cancer.

**Key Message: Consumption of fermented milk of probiotic *Lactobacillus helveticus* MTCC 5463 may help in prevention of colon cancer.**