Evaluation of Anticancer and Antioxidant Effects of Unsaturated Fatty Acids from *Yarrowia lipolytica*: Co-Culture vs. Metabolite-Based Approaches

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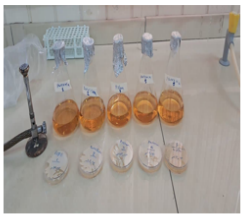
Introduction

Project

This research supports sustainable biotechnology by utilizing *Yarrowia lipolytica*, a GRAS-status, non-pathogenic yeast, as a microbial cell factory for producing health-promoting unsaturated fatty acids. Compared to animal-based or chemically synthesized fatty acids, microbial production offers a low-cost, renewable, and eco-friendly alternative. This approach reduces environmental impact, avoids overfishing (in the case of omega-3 from fish oil), and aligns with green chemistry and circular bioeconomy principles.

Background



Yarrowia lipolytica is a promising oleaginous yeast capable of producing various unsaturated fatty acids (UFAs), including omega-3 and omega-6, which are known for their health-promoting properties. Recent evidence suggests these UFAs may exhibit anticancer and antioxidant activities. This study explores the potential of *Y. lipolytica*-derived UFAs in combating human cancer cells through both direct co-culture and metabolite-based treatments.

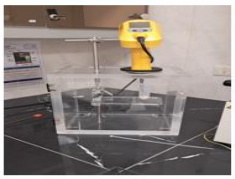
Research Question

Do the unsaturated fatty acids produced by *Yarrowia lipolytica* exhibit significant anticancer and antioxidant effects by reducing the viability of human colon cancer cells (HT-29) and decreasing intracellular reactive oxygen species (ROS), and is there a notable difference between the effects observed in direct co-culture and those from cell-free metabolites?

Material and methods

1. Microorganism and Culture Conditions

- **Strain**: *Yarrowia lipolytica* (laboratory or wild-type strain).
- **Culture medium**: Yeast extract peptone dextrose (YPD) broth or lipid-inducing nitrogen-limited medium.
- **Incubation conditions**: Cultures were incubated at 28–30 °C with agitation at 150 rpm for 72–120 h.
- **Metabolite extraction**: Culture supernatants were obtained by centrifugation at 5000 rpm for 10 min, followed by sterile filtration through 0.22 µm membrane filters.



2. Cell Disruption and Lipid Extraction

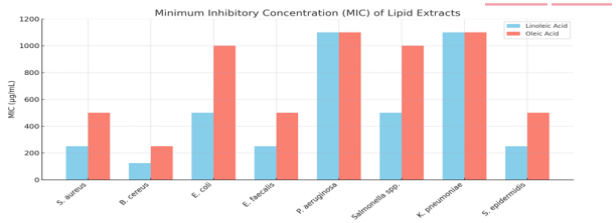
- **Cell disruption**: Yeast biomass was harvested and subjected to homogenization and ultrasonic treatment to break the cell walls and release intracellular lipids.
- **Lipid extraction**: Lipids were extracted from the disrupted biomass using either the Bligh and Dyer method or the Folch method.
- **Solvent removal and concentration**: Crude lipid extracts were purified and concentrated using a rotary evaporator under reduced pressure.

3. FAME Preparation and Combined GC-MS / FTIR Analysis

- **FAME preparation**: Transesterification of extracted lipids was performed using methanolic HCl or NaOH to obtain fatty acid methyl esters (FAMEs).
- **Gas Chromatography–Mass Spectrometry (GC-MS)**: FAMEs were analyzed using GC-MS to determine the fatty acid profile. Separation was performed on a capillary column with helium as the carrier gas, and compounds were identified by comparing retention times and mass spectra with reference standards and NIST library data.

Results

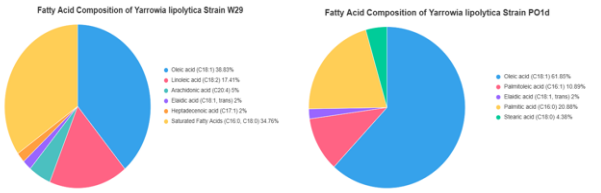
Antimicrobial effects of oleic acid and linoleic fatty acid derived from yarrowia lipolitica



Linoleic acid (blue bars) shows stronger antibacterial activity, especially against Gram-positive bacteria (MIC: 125–250 µg/mL), while oleic acid (red bars) is less effective (MIC: 250–1000 µg/mL). Neither fatty acid fully inhibited *P. aeruginosa* or *K. pneumoniae* up to 1000 µg/mL. This highlights linoleic acid's superior antimicrobial potential.

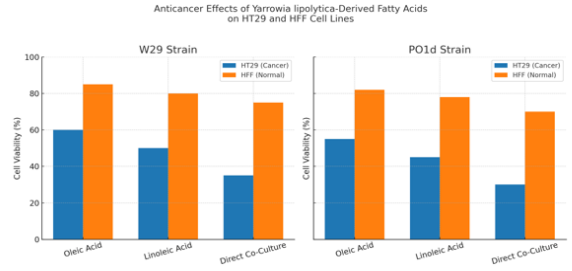
Disscusion

Total lipid content



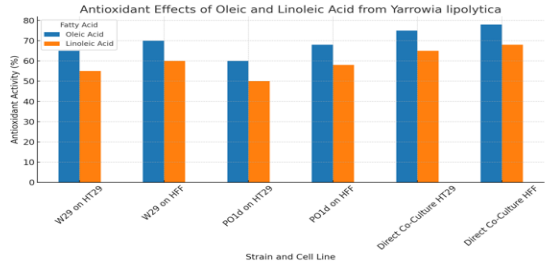
The pie charts compare the fatty acid profiles of *Yarrowia lipolytica* strains W29 and PO1d. Strain PO1d contains a higher percentage of oleic acid (61.80%) compared to W29 (38.83%), indicating a richer monounsaturated fatty acid content. In contrast, W29 shows a more diverse composition, including higher levels of linoleic acid (17.41%) and saturated fatty acids (34.76%). This suggests that PO1d may be more suitable for applications needing high oleic acid content, while W29 offers a broader fatty acid profile.

Anticancer effects of oleic acid and linoleic fatty acid derived from yarrowia lipolitica and Direct Co-Culture



the impact of oleic acid, linoleic acid, and direct co-culture from two strains of *Yarrowia lipolytica* (W29 and PO1d) on HT29 (colon cancer) and HFF (normal fibroblast) cell lines. Lower cancer cell viability alongside higher normal cell viability indicates selective anticancer potential.

Antioxidant effects of oleic acid and linoleic fatty acid derived from yarrowia lipolitica and Direct Co-Culture



Results show that both fatty acids exhibit notable antioxidant activity, with linoleic acid generally performing slightly better. The direct co-culture condition yields the highest antioxidant effects on both cell lines, suggesting potential synergistic effects. Strain W29 also shows higher activity compared to PO1d.

Conclusions

Yarrowia lipolytica-derived linoleic and oleic acids exhibit significant anticancer and antioxidant effects on HT-29 colon cancer cells. Linoleic acid demonstrates stronger antimicrobial activity (MIC: 125–250 µg/mL) than oleic acid (MIC: 250–1000 µg/mL). Direct co-culture with *Y. lipolytica* (especially strain W29) enhances these effects compared to cell-free metabolites, indicating synergistic potential. As a non-pathogenic, GRAS-status yeast, *Y. lipolytica* is an optimal candidate for use as a probiotic and in large-scale biotechnology applications, serving as a sustainable resource for the eco-friendly production of bioactive unsaturated fatty acids for cancer therapy and oxidative stress management.