# Fluorimetric Detection of Cell Membrane Lesions in Wine Lactic Acid Bacteria Exposed to Phenolic Compounds

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# Lactic Acid Bacteria

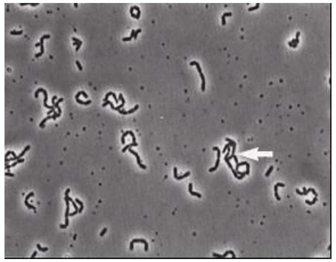
- General characteristics of Lactic Acid Bacteria (LAB):
  - Gram positive, fermentative, aerotolerant bacteria
  - Lactic acid → major product of carbohydrate metabolism
  - Complex nutritional requirements
  - Widely used in food industry – fermented foods

# Lactic Acid Bacteria in grapes and wine

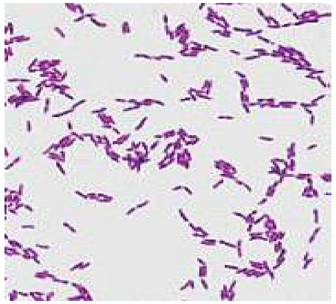
- Role in winemaking:
  - Malolactic Fermentation (malic acid → lactic acid)
  - Increase microbiological stability of wine
  - Improvement of wine aroma

- Negative effects on wine quality:
  - Production of "off-flavours"
  - Bitterness and changes in wine texture
  - Production of potential chemical hazards (*e. g.* biogenic amines)

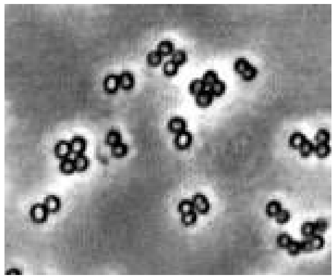
- Origin:
  - Grapes (indigenous microflora), cross-contamination, *starter* cultures



*Oenococcus*



*Lactobacillus*



*Pediococcus*

# Lactic Acid Bacteria in wine

- Wine is an unfavourable environment for LAB growth:
  - High ethanol concentration (9-15%)
  - Presence of sulfur dioxide (up to 150 mg $L^{-1}$)
  - Low pH (2.8-3.8)
  - Low nutrient concentration
  - Natural antibacterial compounds (*e. g.* phenolic compounds)

# Phenolic compounds in grapes and wine

- Responsible for wine colour, astringency and bitterness
- Origin: Grapes (seeds, skin, stalk and pulp) and wood (barrels)
- Total concentration in wines ~ 2-3 g/L
- Antioxidant and antimicrobial properties
- Includes tannins, flavonoids, phenolic acids and aldehydes



# Phenolic acids and aldehydes

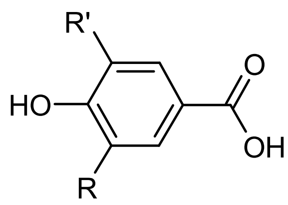
## Phenolic acids

- Mainly present in grape skin and pulp
- Weak organic acids (pKa ≈ 4.2-4.5)
- Concentration in wine: 100-200 mg $L^{-1}$

## Phenolic aldehydes

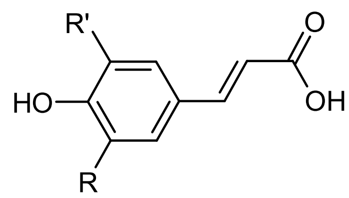
- Mainly derived from oak wood
- Important aromatic compounds
- Concentration in wine: 0-5 mg $L^{-1}$

**Benzoic acids**



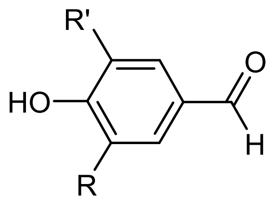

*p*-Hydroxybenzoic (R=R'=H)
Protocatechuic (R=H; R'=OH)
Gallic (R=R'=OH)
Vanillic (R=H; R'=$H_3CO$)
Syringic (R=R'=$H_3CO$)

**Cinnamic acids**



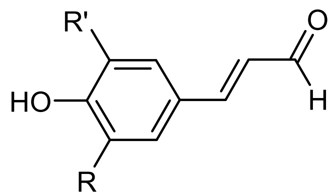

*p*-Coumaric (R=R'=H)
Caffeic (R=H; R'=OH)
Ferulic (R=H; R'=$H_3CO$)
Synapic (R=R'=$H_3CO$)

**Benzaldehydes**




*p*-Hydroxybenzaldehyde (R=R'=H)
Dihydroxybenzaldehyde (R=H; R'=OH)
Trihydroxybenzaldehyde (R=R'=OH)
Vanillin (R=H; R'=H3CO)
Syringaldehyde (R=R'=H3CO)

**Cinnamaldehydes**



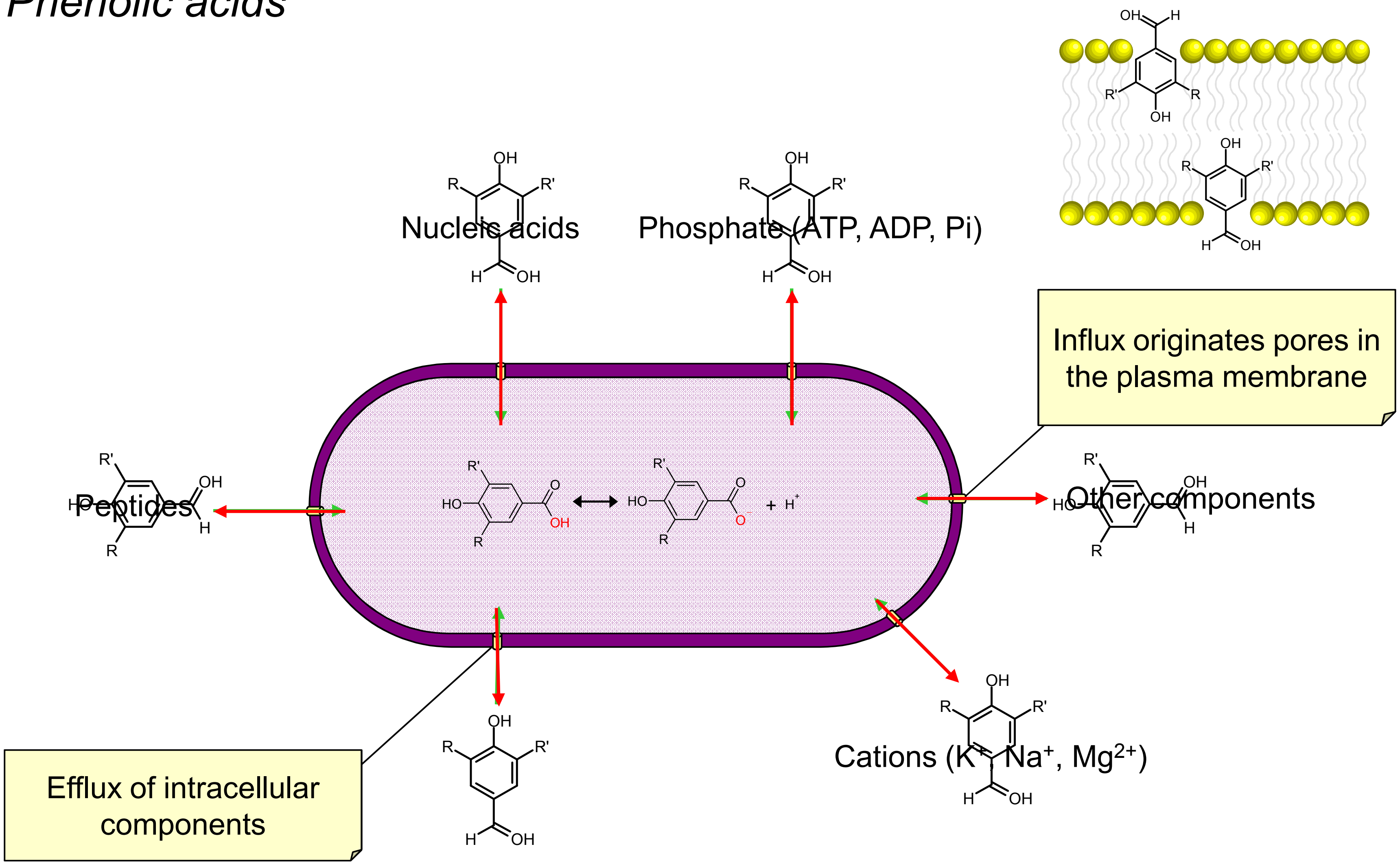

*p*-Hydroxycinnamaldehyde (R=R
Coniferaldehyde (R=H; R'=H3C(
Synapaldehyde (R=R'=H3CO)

# Phenolic acids and aldehydes

- Antibacterial activity
- Inactivation mechanisms not yet fully understood:
  - Membrane-active compounds:
    - Create pores in lipidic membrane
  - Cytoplasmic poisons
    - React with proteins/enzymes
    - Lower intracellular pH (phenolic acids)

# Effect on cytoplasmic membrane

*Phenolic acids*



# Methods of evaluating membrane damages

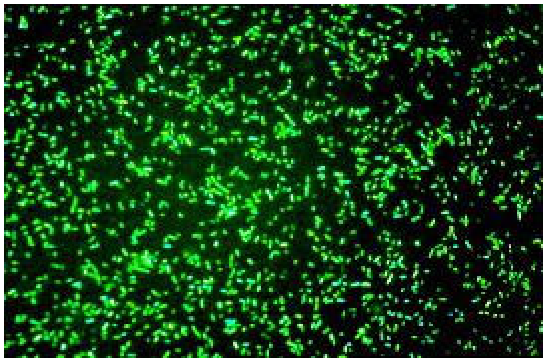
1) Efflux of cell constituents:
   - Nucleic acids and peptides (UV spectroscopy)
   - Cations (Potentiometry,Atomic Absorption Spectroscopy, …)
   - ATP / Phosphate (UV/VIS spectroscopy, Bioluminescence)

2) Chemical probes
   - Analyze recovery of cells in growth medium with NaCl after sub-lethal exposure to the chemical stress

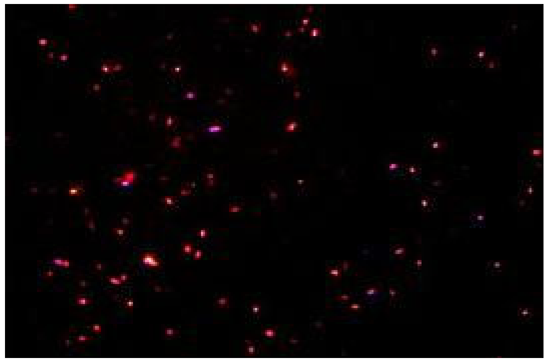
3) Fluorimetric probes
   - Use different probes for damaged and undamaged cells

# Fluorimetric detection of membrane damage

- Commercial fluorescence kit – BacLight™
- Mixture of 2 dyes which react with nucleic acids:
  - SYTO® 9 – penetrates both undamaged and damaged cells
  - Propydium iodide (PI) – penetrates damaged cells only, quenching SYTO® 9 fluorescence



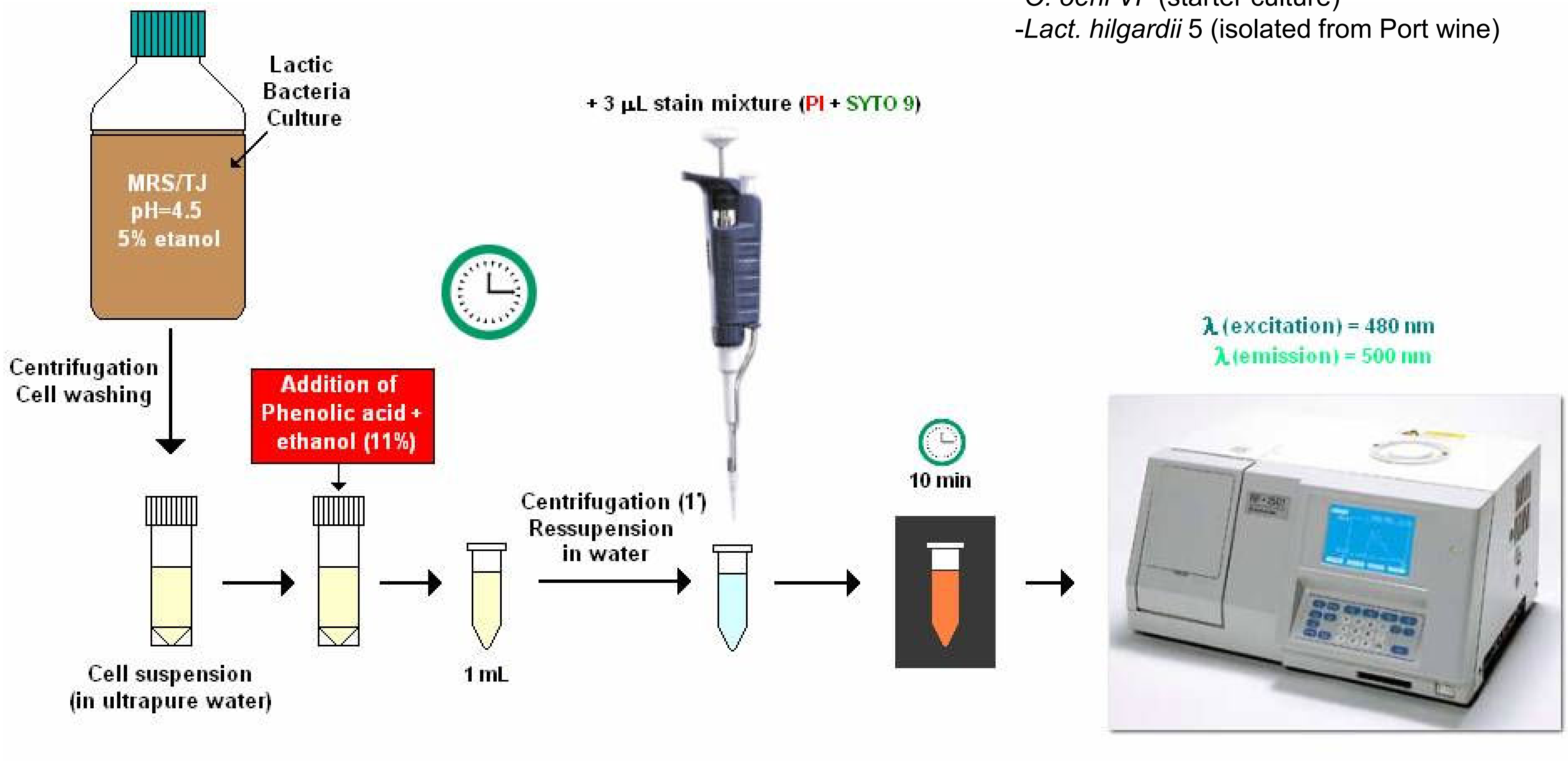
*Viable Cells*



*Damaged Cells*

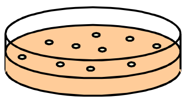
# Fluorimetric detection of membrane damage

Tested strains:
-*O. oeni VF* (starter culture)
-*Lact. hilgardii* 5 (isolated from Port wine)

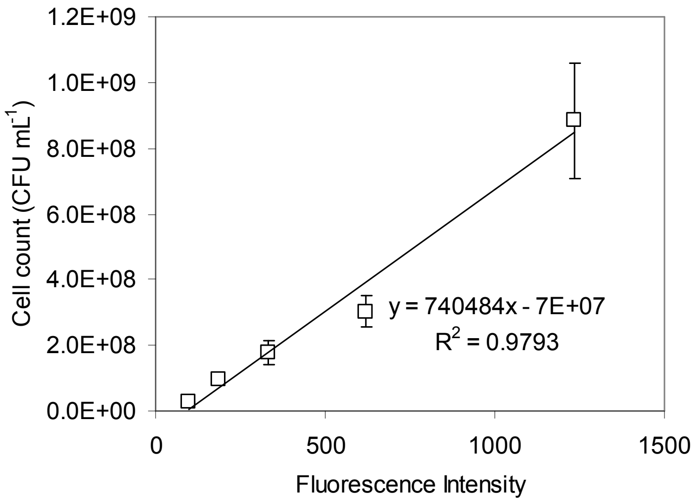


# Fluorimetric detection of membrane damage

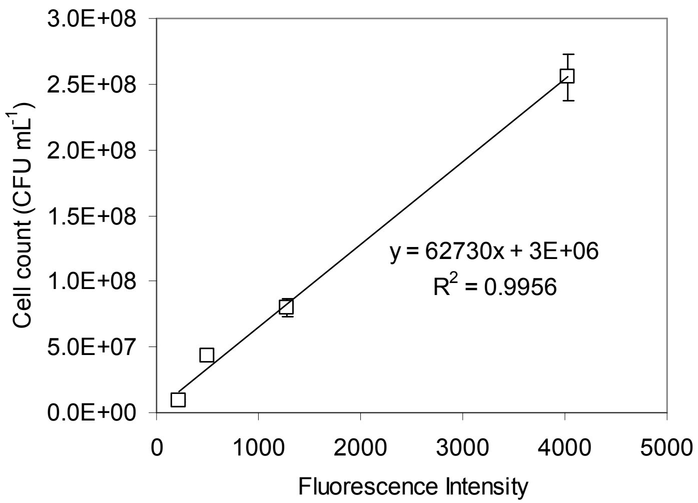
<u>Calibration curves</u>
(Fluorescence Intensity vs. Plate counts)



*Lactobacillus hilgardii 5*

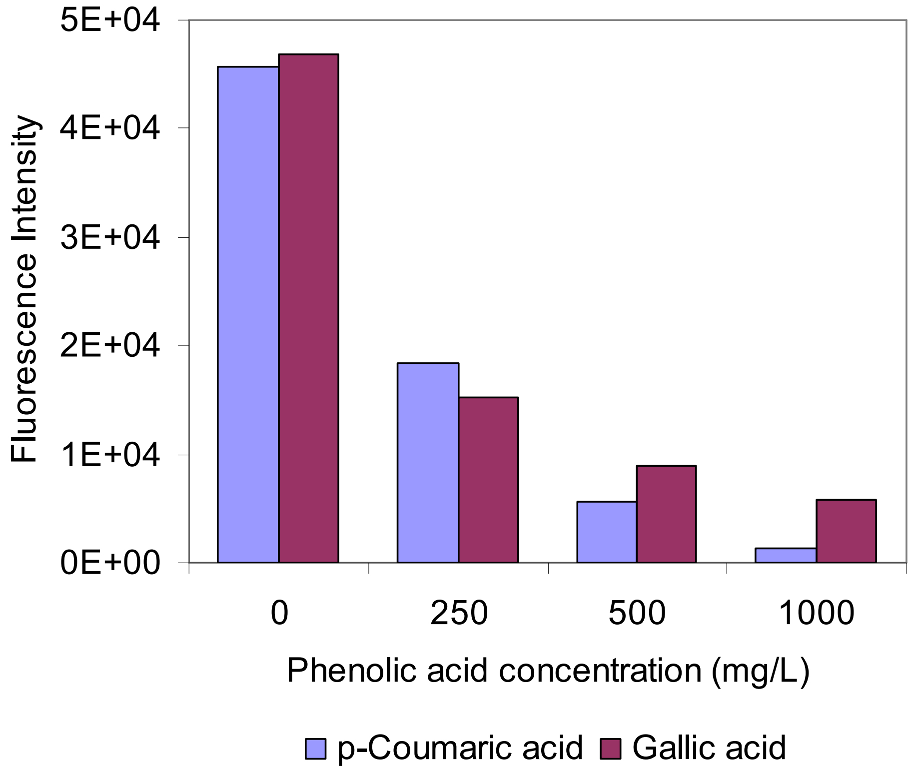



*Oenococcus oeni VF*

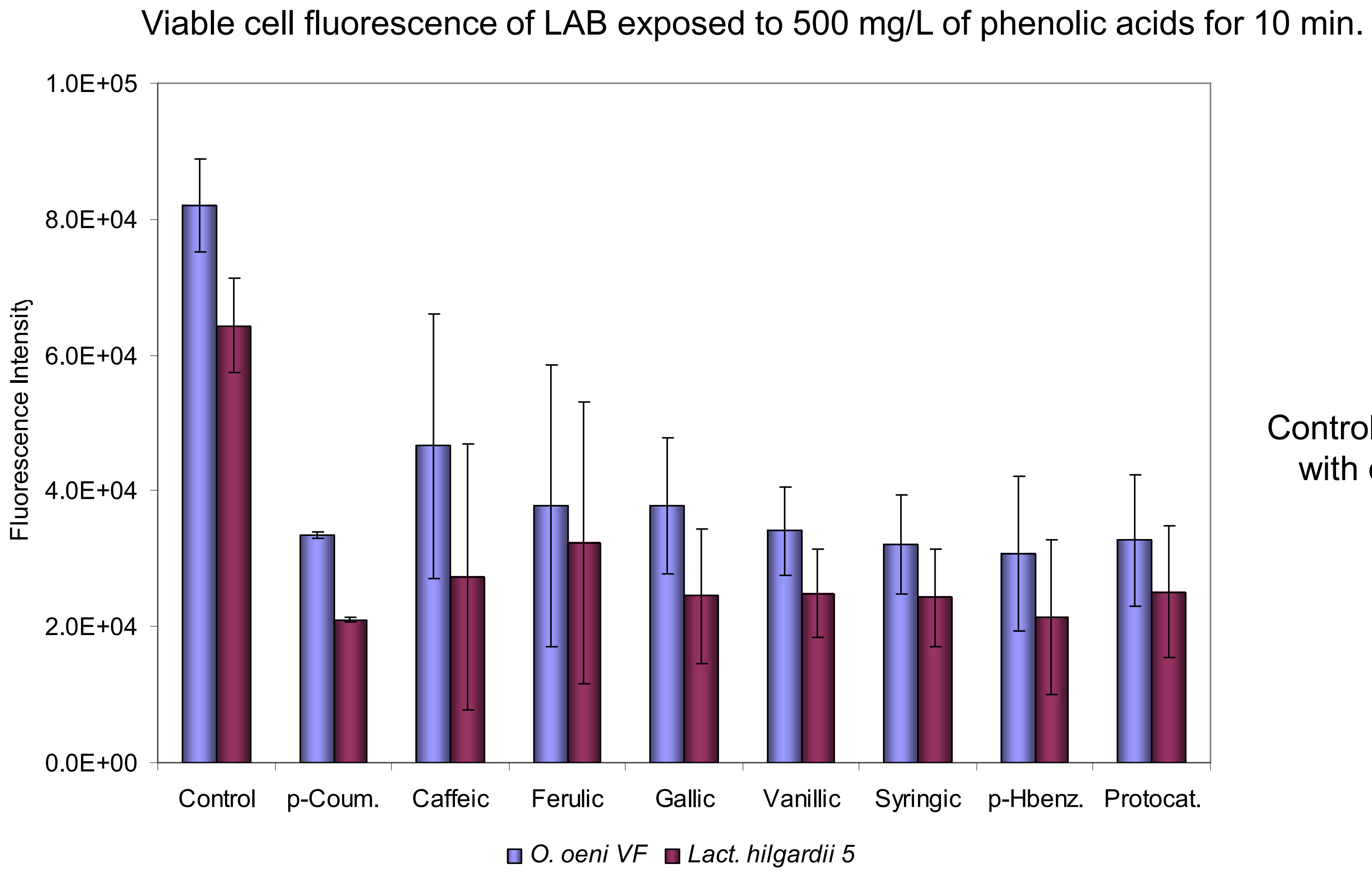



# Fluorimetric detection of membrane damage

Viable cell fluorescence of *Lact. hilgardii 5* after a contact period of 10 minutes



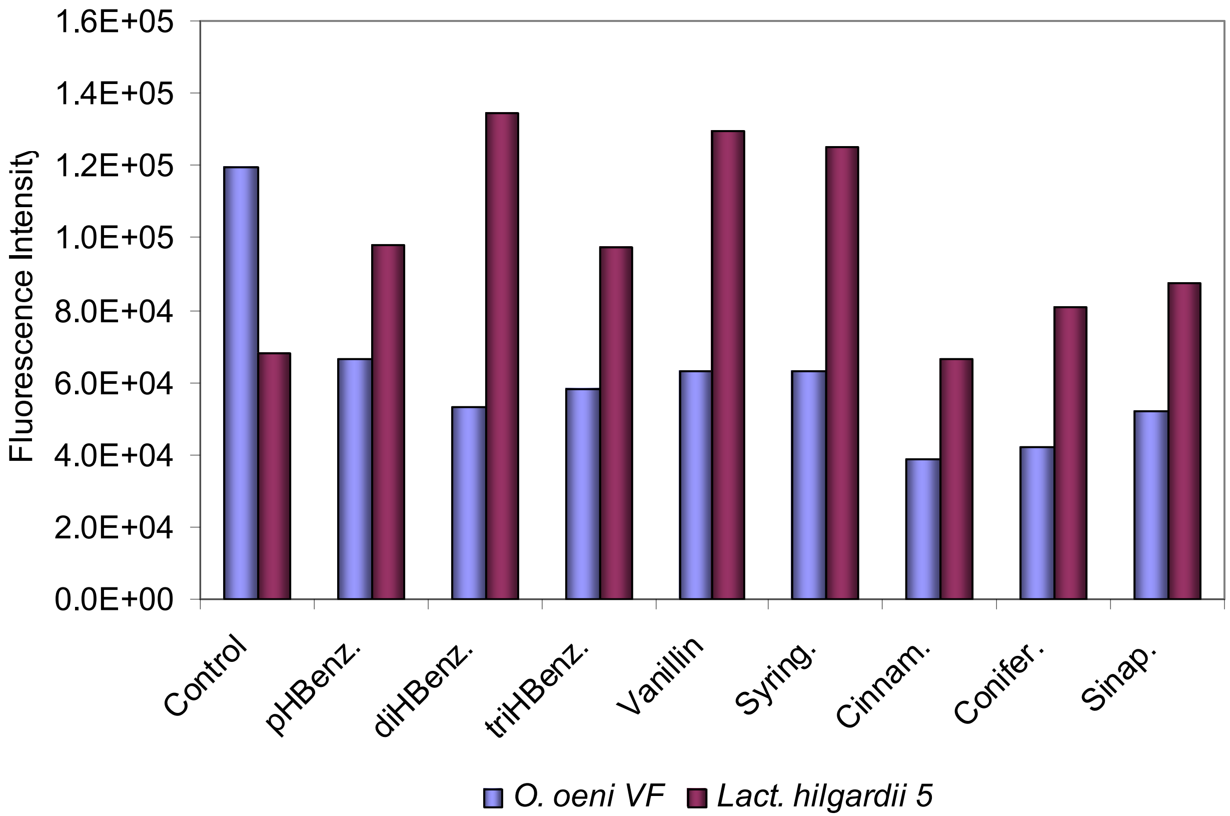
# Fluorimetric detection of membrane damage




Control = experiment with ethanol only

# Fluorimetric detection of membrane damage

Viable cell fluorescence of LAB exposed to 1000 mg/L of phenolic aldehydes for 10 min.




Control = experiment with ethanol only

# Conclusions

- Phenolic acids increased membrane permeability of both tested strains of lactic acid bacteria.
- Phenolic aldehydes increased membrane permeability in *O. oeni* VF.
- In the case of *Lact. hilgardii* 5, some phenolic aldehydes had an apparent protective effect against ethanol toxicity – the same result was obtained in $K^+$ efflux experiments.
- The comercial fluorescence kit used was appropriate for detection of membrane lesions in LAB caused by phenolic acids and aldehydes.

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***Thank you for your attention!***

For more information please contact: fmcampos@mail.esb.ucp.pt

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