



PEF4Alg Biotics

Valorization of microalgal extracts obtained by pulsed electric field in lactic acid fermentation

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Microalgae

Lipids

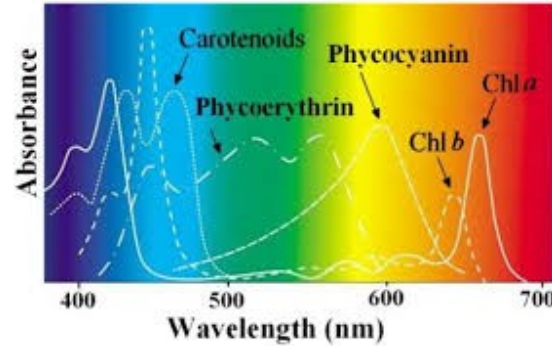
- Polyunsaturated fatty acids

Chlorella vulgaris



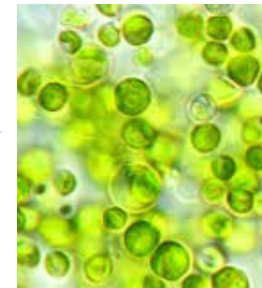
1000 L photobioreactor, KIT

Pigments



Proteins

CO₂
minerals
H₂O

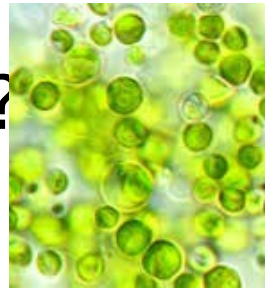


Biodiesel

Not enough!

More products to make it economically viable

How to get more from *C. vulgaris* biomass?

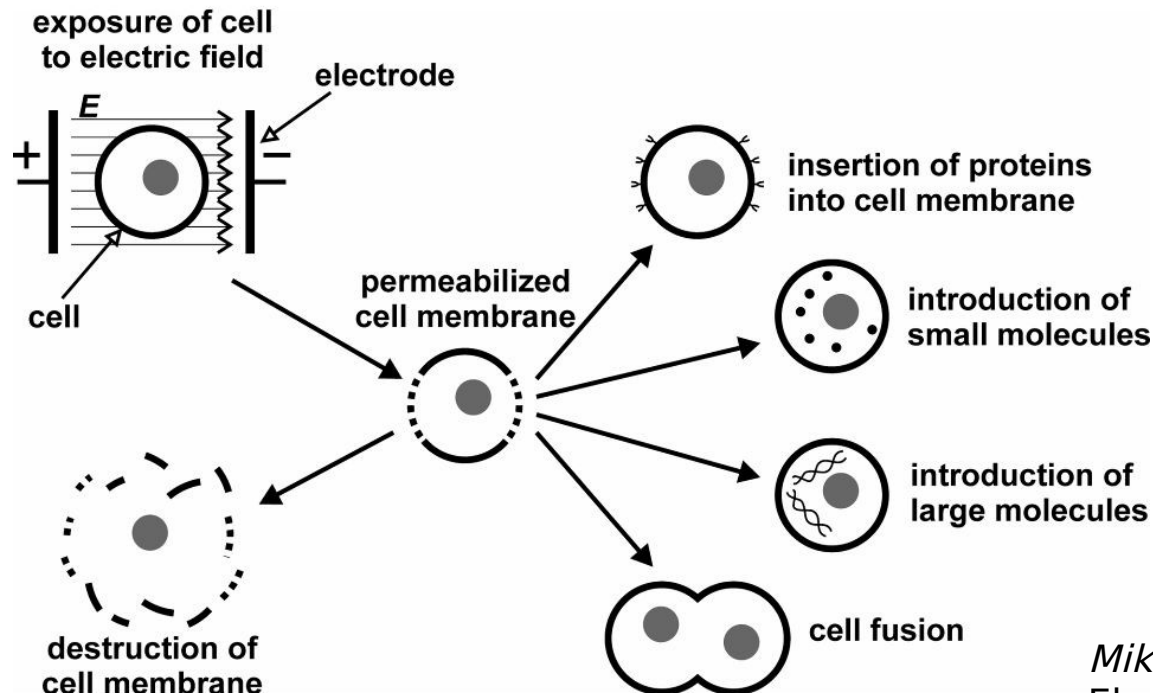


Pulsed Electric Field - PEF treatment

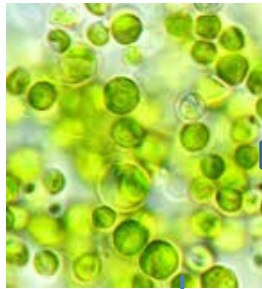
- ✓ of adequate strength and duration
- ✓ on eukaryotic and prokaryotic cells
- ✓ causing increase in cell membrane permeability, if increase in transmembrane voltage surpasses certain value



Institute for Pulsed Power and Microwave Technology



Miklavčič, 2014,
Electroporation based
technologies and treatments



PEF4Alg Biotics

Lactobacillus rhamnosus
Lactobacillus paracasei

1. **Valorization of released components**

Nutrients recycling

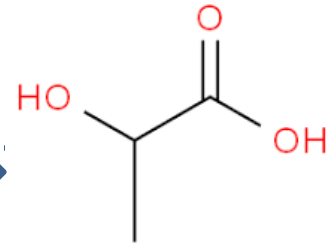
Release of small water soluble components



2. **Valorization of proteins**

Water soluble proteins

FERMENTATION

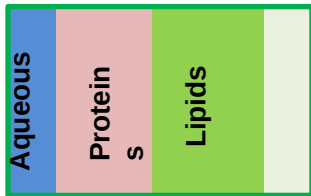


lactic acid + probiotics

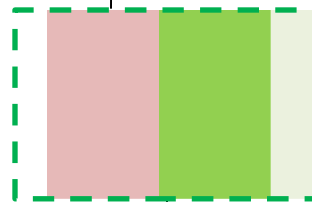
3. **Biofuel**

Lipids

Microalgae biomass



PEF treatment



Ethanol extraction

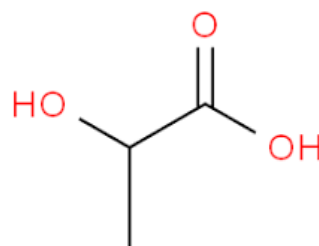


Anaerobic digestion

Thermo-chemical conversion

PEF obtained aqueous fraction of microalgae

Lactobacillus rhamnosus
Lactobacillus paracasei



lactic acid

As chemical



As preservative



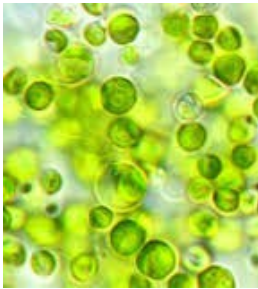
As monomer for poly(lactic) acid



Content of proteins in PEF obtained extracts

C. vulgaris suspension was concentrated (of approx. 0.8 g/l)

Centrifugation: 10000 g, 6 minutes and resuspended – adjusting conductivity



PEF treatment

- Continuous treatment,
- 10 kV/cm, 100 ns, 4.5 s⁻¹
- 20 h incubation, 23 °C



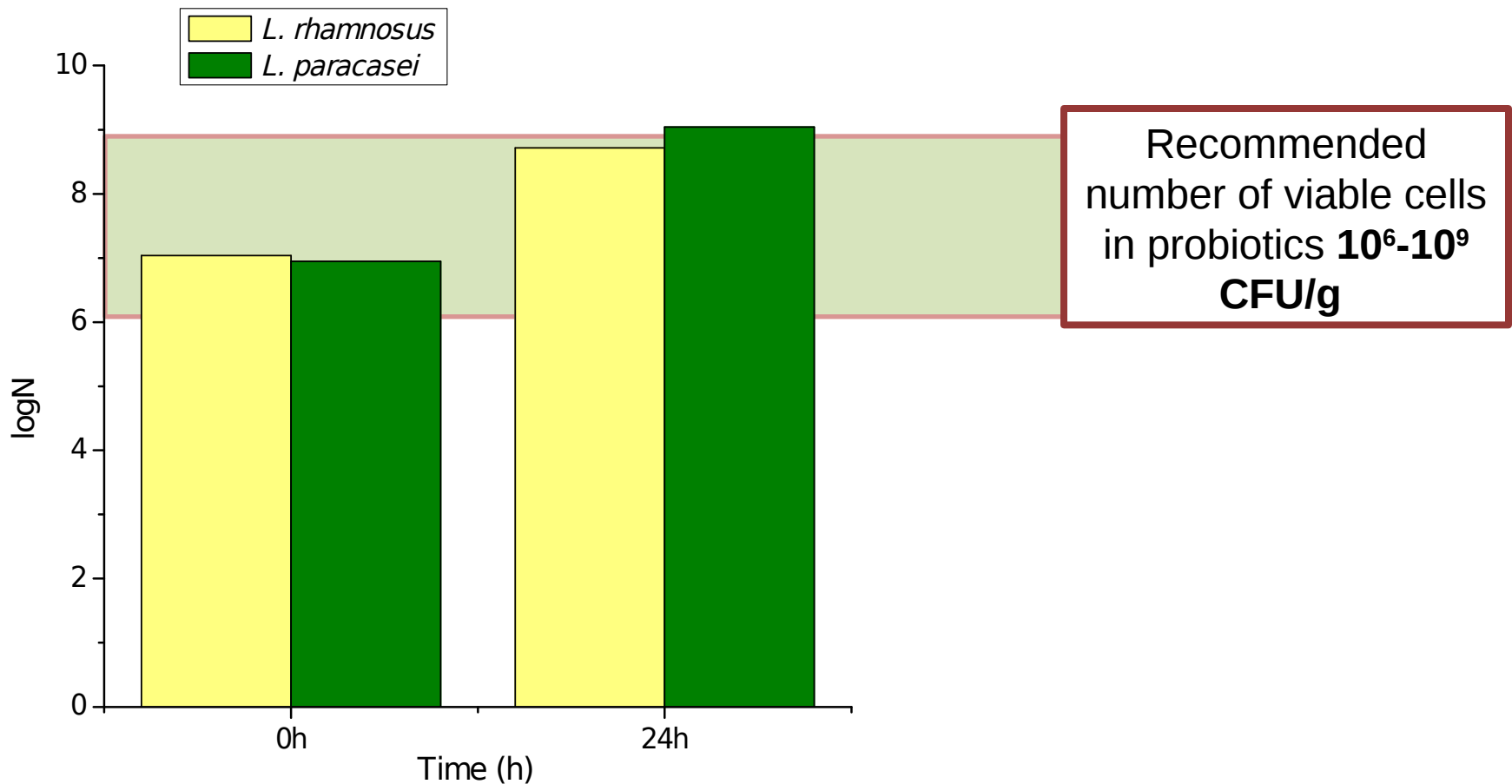
- **20-23% of proteins extracted**
- **Protein concentration: 0.950 g/L (by Lowry)**
- **Role of C/N ratio for lactic acid fermentation**



Supplementation with fermentable sugars

FERMENTATION

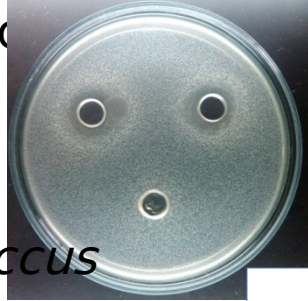
- Growth of *L. rhamnosus* and *L. paracasei* on extracts



Antimicrobial activity of

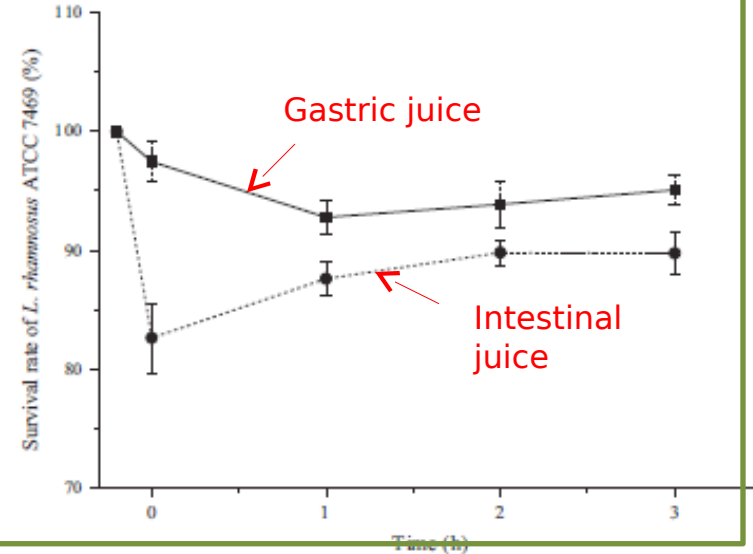
Against:

- *Salmonella enteritidis*
- *Staphylococcus aureus*
- *Shigella sonnei*



Important probiotic characteristics of selected bacteria

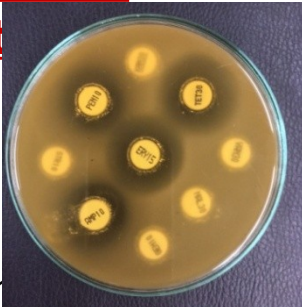
Resistance to gastrointestinal stress (low pH, pepsin, pancreatin, bile salts)



Antibiotic resistance

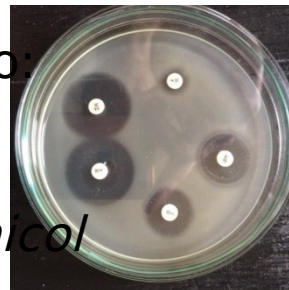
Resistance to:

- *Gentamicin*
- *Kanamycin*
- *Streptomycin*
- *Vancomycin*



Susceptibility to:

- *Erythromycin*
- *Tetracycline*
- *Chloramphenicol*
- *Penicillin G*
- *Ampicillin*



Antioxidant activity of

- intact cells
- intracellular cell free extracts

Lactic acid fermentation

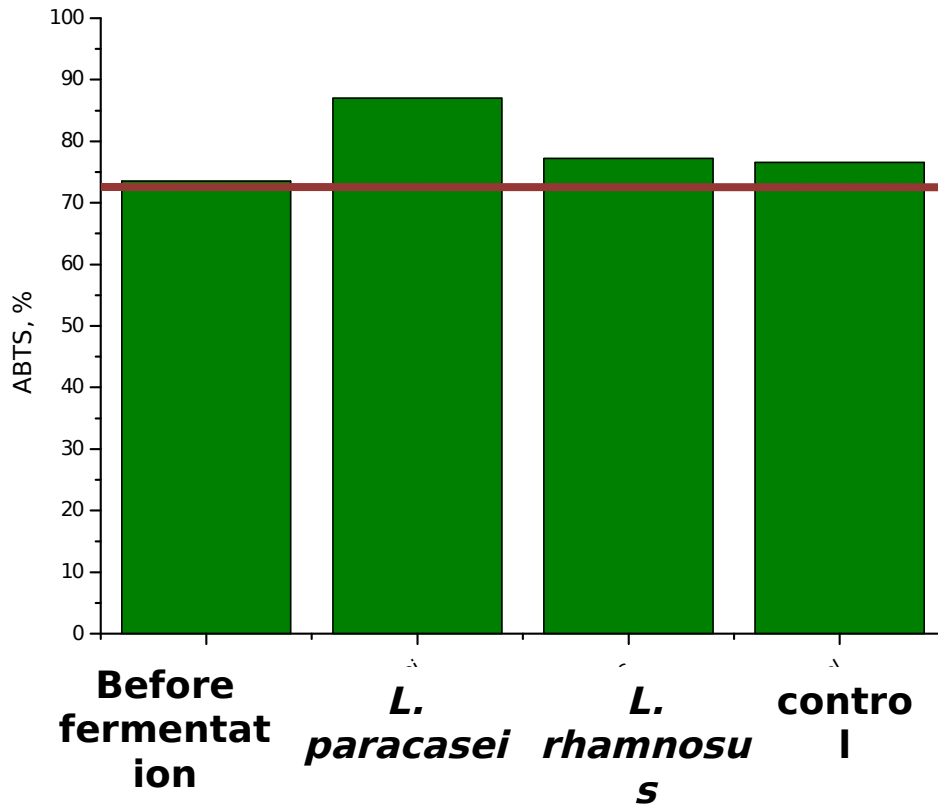
- Batch open fermentation – avoiding thermal treatment
- 37 °C, 24h, 100 rpm
- 5% (v/v) inoculum concentration
- ~30 g/L initial sugar concentration

Strain	LA concentration	LA yield	LA productivity	Stereoselectivity
<i>L. rhamnosus</i>	24.24 g/L	85%	1.01 g/Lh	> 97% L-lactic acid
<i>L. paracasei</i>	27.3 g/L	91%	1.14 g/Lh	> 97% L-lactic acid

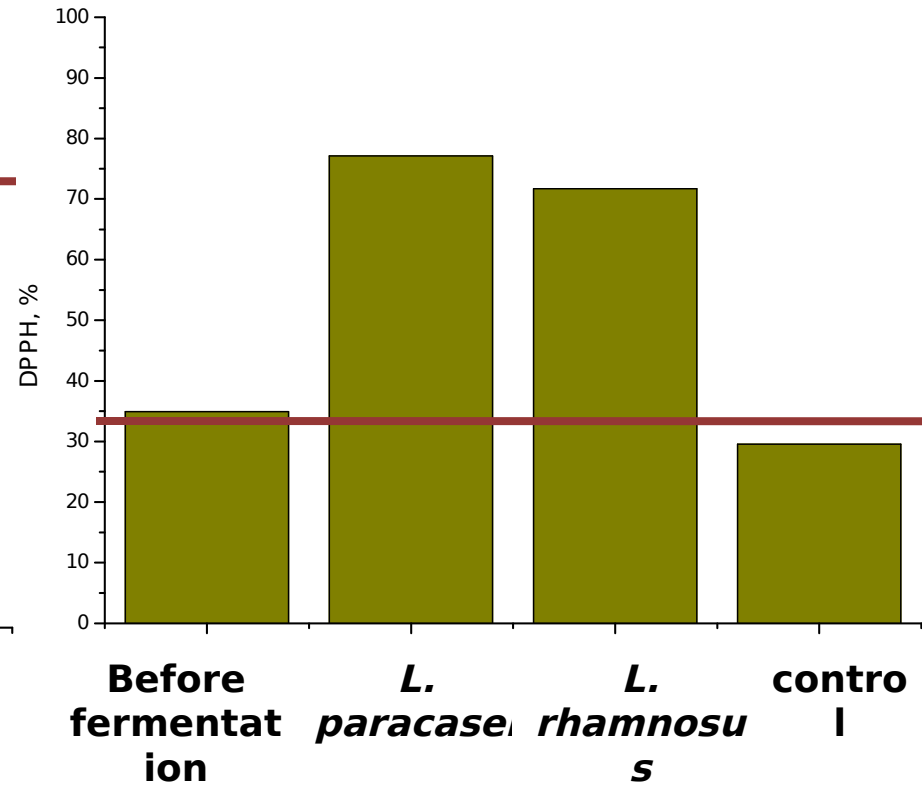
Not significant changes were observed in free amino nitrogen concentration!

Antioxidant activity

ABTS



DPPH



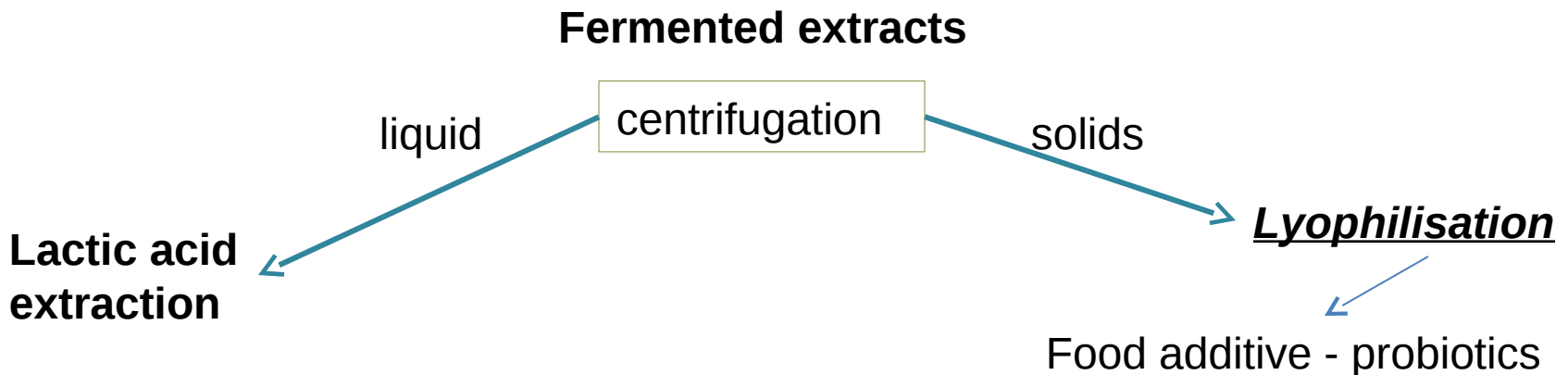
Revalorisation of PEF obtained extracts

Chlorella vulgaris – FDA approved for human nutrition

L. rhamnosus

L. paracasei – GRAS status

- ✓ Can be performed in open fermentation mode – avoiding thermal sterilisation or filtration
- ✓ But... changes in colour, oxidation, temperature, pH ...
- ✓ After the LA fermentation high antioxidant activity in fermented extracts





Ministry of Education, Science
and Technological Development of
Republic of Serbia



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DAAD

Deutscher Akademischer Austauschdienst
German Academic Exchange Service

Thank you for your attention!

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