

Novel Protective Cultures for Clean-Label Food Products

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State of the Art

In the post CoViD pandemic era, great attention is paid to the sustainability and to the food products safety (Pires et al. 2021). In addition, the emergency of antibiotic-resistant pathogens and consumers demand for minimally processed foods, highlight the need to adopt “clean label approaches” as screens against foodborne spoilers and pathogens (Heymich et al. 2021). In the control of foodborne pathogens, several authors (Hammani et al. 2019; Mota-Gutierrez and Cocolin, 2021), reported a great number of protective lactic acid bacteria strains which commonly produce organic acids, peroxides, enzymes and bacteriocins. To date, the antagonistic action of commercial protective cultures is mainly due to the production of bacteriocins. However, the efficiency of their application as food additives may be limited for various reasons, particularly for the pH-depend effectiveness. On highlights bases, novel protective cultures were developed. Particularly, new multi-strain cultures with improved performance over a wide pH range were selected.

The main goal of the R&D Project is the development and validation of new multi-strain cultures with improved performance over a wide pH range were selected.

Experimental Design

Isolation identification

- From with harsh environmental conditions;
- Specie level identification
- Sequencing

Primary screening

Effect on undesirable microorganisms

- Agar Well Diffusion Assay

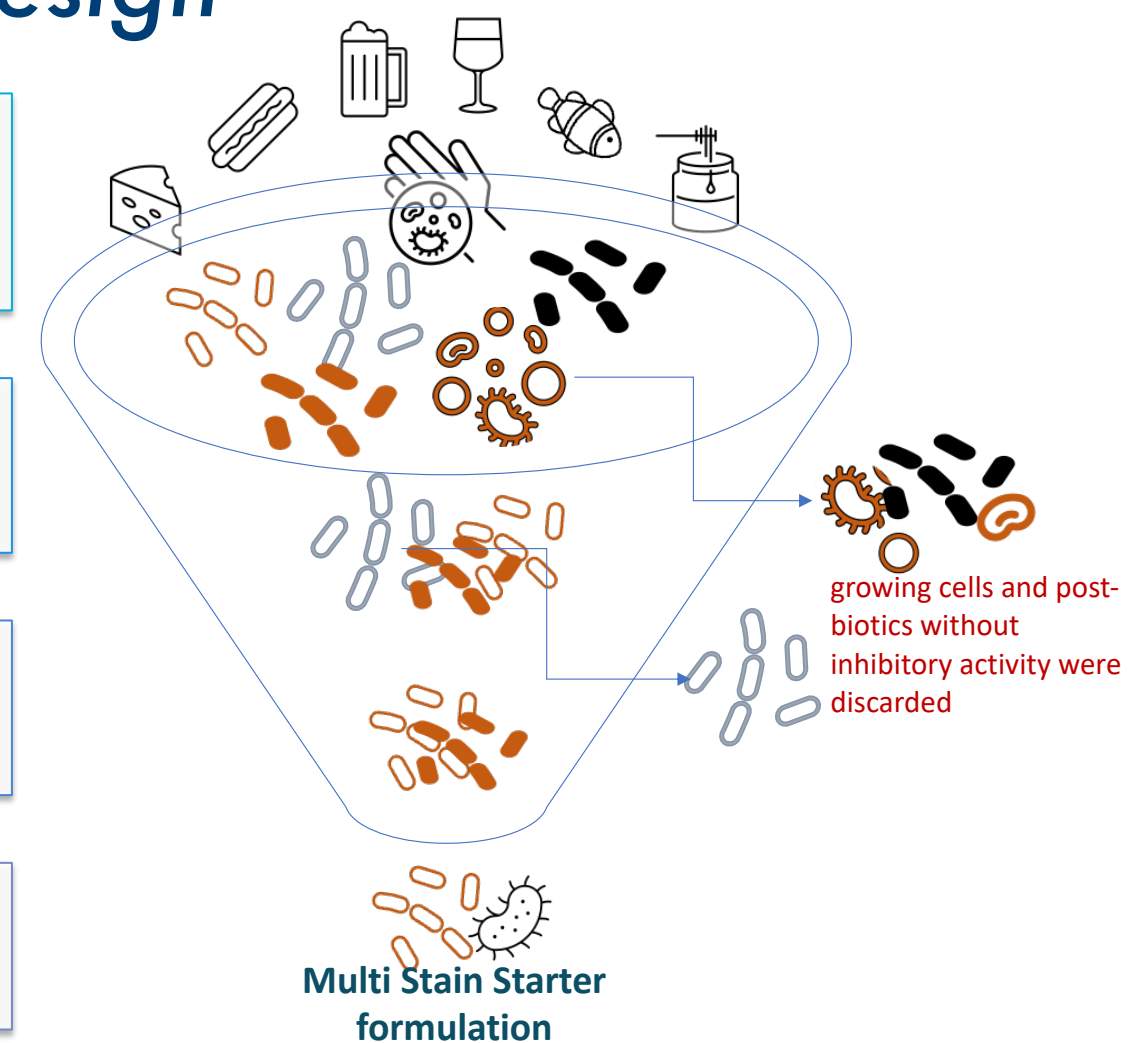
Secondary screening

Effect on undesirable microorganisms at different pH

- Survival model and polynomial equations

Cultures Formulation

Data analysis and multi strain starter cultures formulation



Results

Producer strains highlighted different effects against the indicator strains.

Based on the inhibitory activity expressed by growing cells, producers were grouped into three clusters (Figure 1).

Most of the strains were grouped in cluster A and showed a low or no antimicrobial activity.

Cluster B grouped 3 strains belonging both to *Latiplantibacillus plantarum* (2) and *Lacticaseibacillus rhamnosus* (1). Finally, 6 strains, belonging to *Lp. plantarum* (4) and to *Lacticaseibacillus paracasei* (2) were grouped in cluster C. These strains produced a moderate or high inhibitory activity against all assayed indicators (except clostridium species).

Only one strain (*Lp. plantarum* grouped in cluster A) produced a strong inhibitory activity against *Clostridium tyrobutyricum*

Four strains were selected: three strains (*Lp. plantarum* LPUM1, for the wide range of antimicrobial activity and one for anti *C tyrobutyricum*.

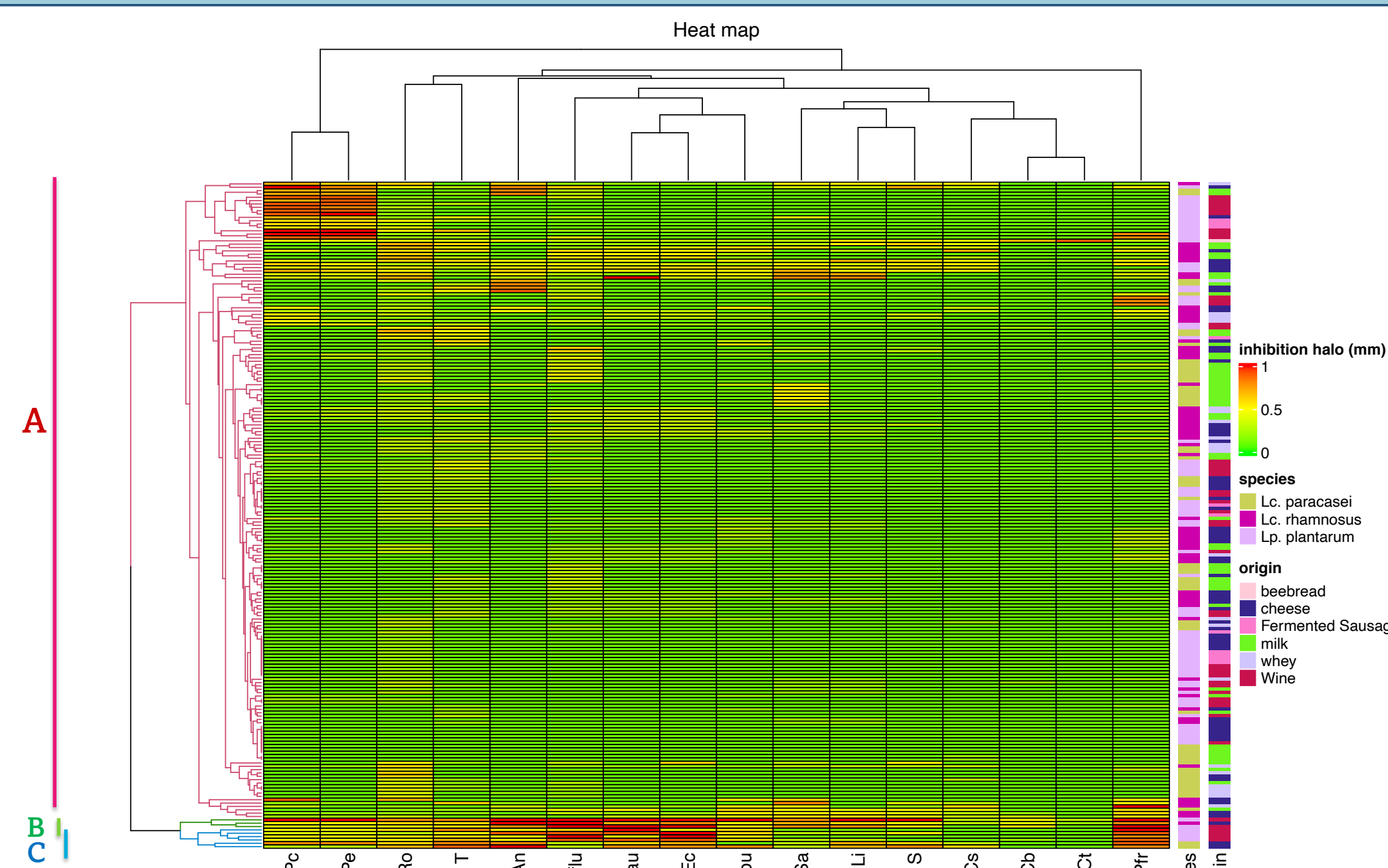


Figure 1. Heat map showing the similarity in the profiles of antimicrobial activity of growing cells of producer's strains vs indicators strains

Penicillium commune (Pc), *Penicillium echinulatum* (Pe), *Pseudomonas fragi* (Pfr), *Tricoderma* (T), *Rizopus oryzae* (Ro), *Clostridium tyrobutyricum* (Ct), *Pseudomonas fluorescens* (Pflu), *Escherichia coli* (Ec), *Pseudomonas aeruginosa* (Pau), *Clostridium butyricum* (Cb), *Staphylococcus aureus* (Sa), *Listeria innocua* (Li), *Pseudomonas putida* (Ppu), *Salmonella* (S), *Clostridium sporogenes* (Cs), *Aspergillus niger* (An)

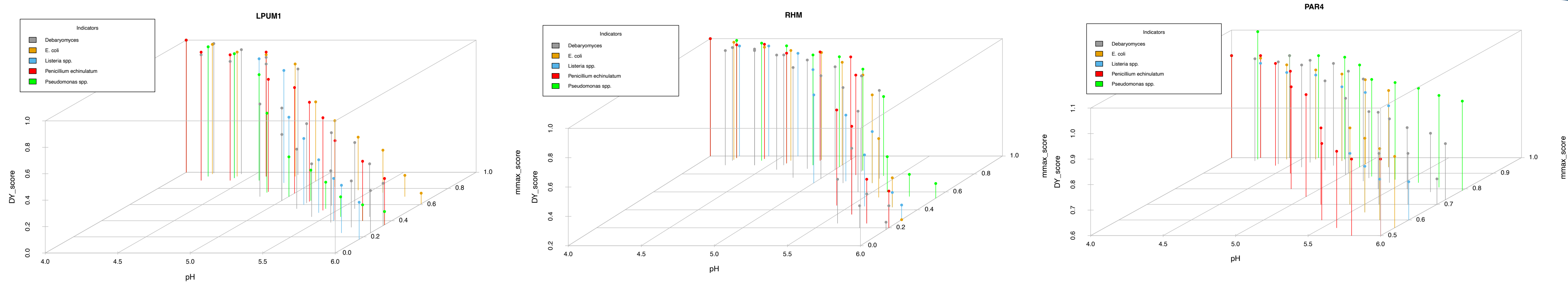


Figure 2. 3D Scatterplot representing growth kinetic parameters (maximum specific growth rate score, μ_{max} _score; decrease in microbial load, D_{Yscore}) of indicators strains at different pH and exposed to *Lp. plantarum* LPUM1, *Lc. rhamnosus* RHM, *Lc. paracasei* PAR4.

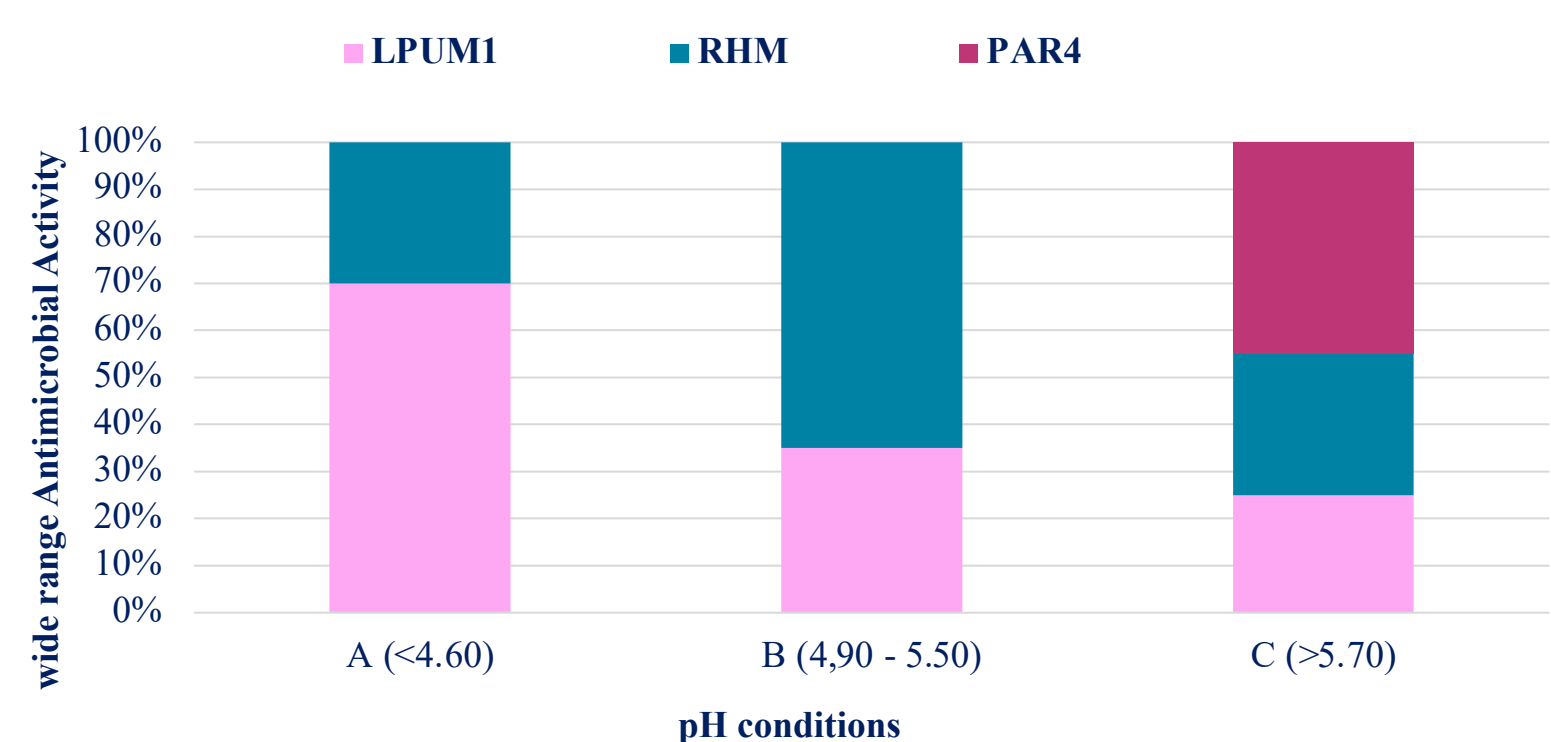


Figure 3. Multi strains cultures antimicrobial activity in different pH conditions

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