Selection of Antifungal Lactic Acid Bacteria Combinations in Dairy Models and Antifungal Molecule Identification

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Context

Dairy products are susceptible to acid-tolerant fungal contaminants

Consequences: Food waste & Economic losses

Solution: Antifungal cultures are of growing interest as an alternative to chemical preservatives or a complement tool to hurdle technologies

Objective

To characterize the antifungal activity of lactic acid bacteria combinations as adjunct cultures in dairy models

Strategy & Results

Screening Strategy

- Screening in 24 well plates
- 2 models: mimicking Cheese & Yogurt
- Strains added as adjunct cultures along with the acidifying starter

Lactobacillus spp.
Leuconostoc spp.
Propionibacterium spp.

1st screening: 32 Strains
- 1 acidifying starter/model

2nd screening: 12 Strains
- 2 acidifying starter/model

Tested in combinations: 5 Strains

10 binary and 3 ternary combinations

Screening of the antifungal activity led to the selection of 5 antifungal strains

13 combinations with the 5 selected lactic acid bacteria

L. harbinensis
L. plantarum
L. rhamnosus

3 binary combinations selected because of their higher antifungal activity than the strains alone

Action spectrum tested against 10 spoiling fungi
- Mucor racemous
- Penicillium commune
- Yarrowia lipolytica
- Galactomyces geotrichum
- Penicillium bialowiezense
- Phoma pinodella
- Candida parapsilosis
- Rhodotorula mucilaginosa
- Meyerozyma guilliermondii
- Trichosporon asahii

Antifungal score: 3: total inhibition
0: no inhibition

L. brevis
L. plantarum
A2
X
Y

Example of the antifungal activity against 4 fungal targets in cheese model for 2 A2 strains (individually or combined) in comparison with 2 commercial antifungal cultures (X and Y)

The 3 combinations improved both antifungal activity and action spectrum

The 3 combinations showed a higher antifungal activity than commercial cultures against the most resistant targets: Y. lipolytica and G. geotrichum

The 3 combinations had a broad inhibition spectrum: 6-9 fungi of the 10 tested

The 3 binary combinations (A1, A2 & A3) exhibited high antifungal activity and broad action spectrum

Metabolomics using 4 chromatographic methods

HPLC-UV/RI Organic Acids (OA)
LC-MS+ for Organic Acids (OA)
GC-FID → for Fatty Acids (FFA)
GC-MS → for Volatile Compounds (VC)

Cheese

Yogurt

4 OA
6 OA
3 OA
4 FFA
3 VC
2 FFA
2 VC

Antifungal molecules produced in significantly higher amounts by the 3 tested combinations in comparison to the controls

Different antifungal molecules produced in the tested models rises the question of the matrix effect

Overall, 23 antifungal molecules produced in higher concentrations in the presence of at least 1 of the 3 selected antifungal combinations, compared to the control models

Conclusions

The screening strategy was successful to select antifungal cultures active in dairy matrices. The 3 selected combinations and their metabolites are natural antifungal alternatives of interest for the dairy industry.

Perspectives

The observed matrix-dependent effect shows the importance of in situ tests. Thus, challenge-tests are in process to determine the antifungal activity of the selected combinations in pilot-scale dairy products.