Foods 2024 Conference
The 5th International Electronic Conference on Foods
28–30 October 2024 | Online
MDPI

POZNAŃ UNIVERSITY OF LIFE SCIENCES

Exploratory survey of qPCR-HRM potential in differentiating *Saccharomyces cerevisiae* var. *boulardii* in probiotic-enriched matrices

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BACKGROUNG & AIM

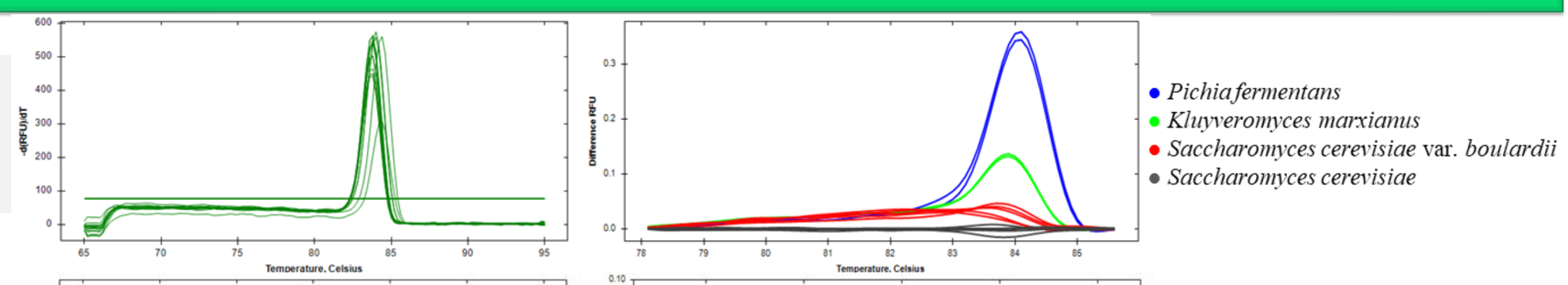
SACCHAROMYCES CEREVISIAE VAR. *BOULARDII* → PROBIOTIC WITH PROVEN HEALTH-PROMOTING EFFECTS → BECAME A COMMON PANACEA FOR LIFESTYLE DISEASES → GROWING NUMBER OF AVAILABLE PROBIOTIC-ENRICHED FOOD → ENVIRONMENT CONDUCIVE TO FOOD ADULTERATIONS → THE NEED FOR RAPID TESTING AND VERIFICATION

METHOD

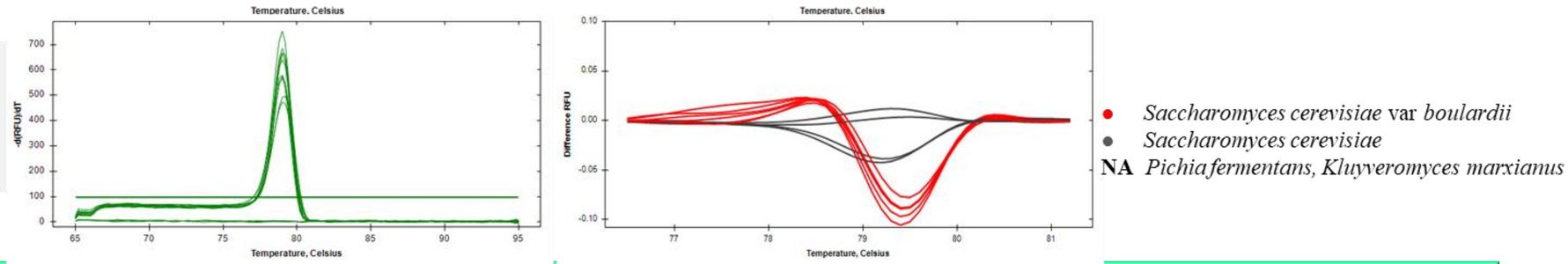
qPCR-HRM ANALYSIS WITH **INTERSPECIES (*18SrRNA*, ITS)**

AND **INTRASPECIES (*HO*, *RPB2*)** PRIMER PAIRS DESIGNED TO AMPLIFY **HETEROGENEOUS REGIONS**

18SrRNA



HO

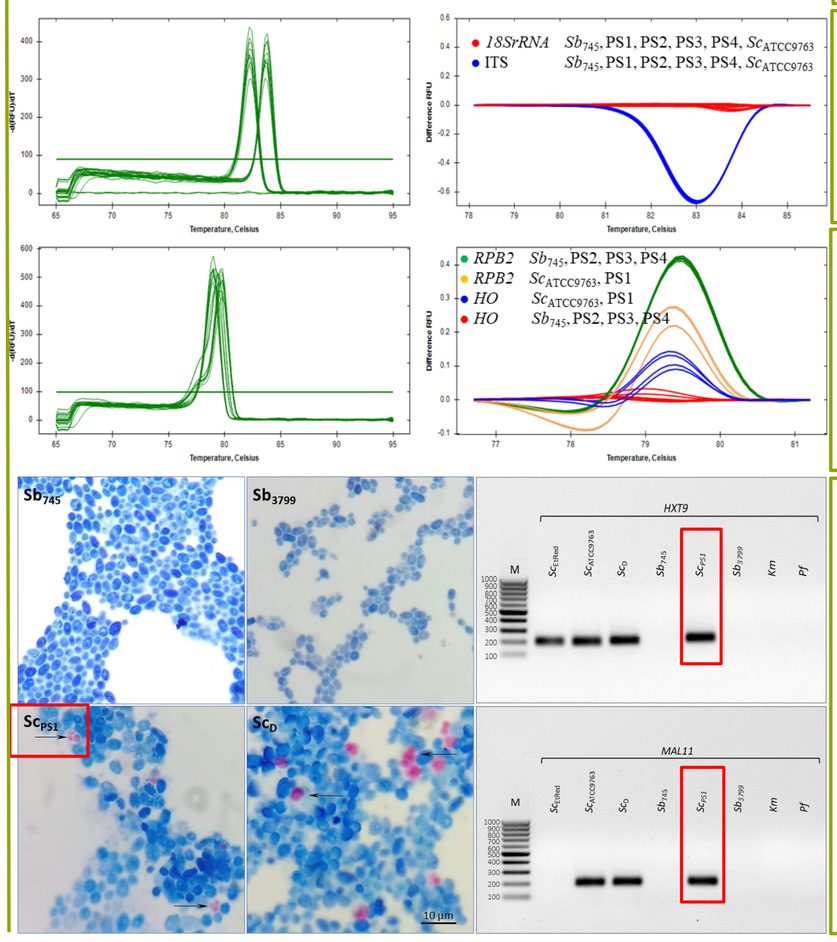


RESULTS

SINGLE YEAST DIETARY SUPPLEMENTS

LABEL	*COMPOSITION*
PS1	*Saccharomyces cerevisiae*
PS2	*Lactiplantibacillus plantarum*, *Bifidobacterium breve*, *Saccharomyces boulardii*
PS3	*Saccharomyces boulardii* and fructooligosaccharides
PS4	*Saccharomyces boulardii*, *L. rhamnosus* GG, fructooligosaccharides

COMPOSITION OF TESTED DIETARY SUPPLEMENTS



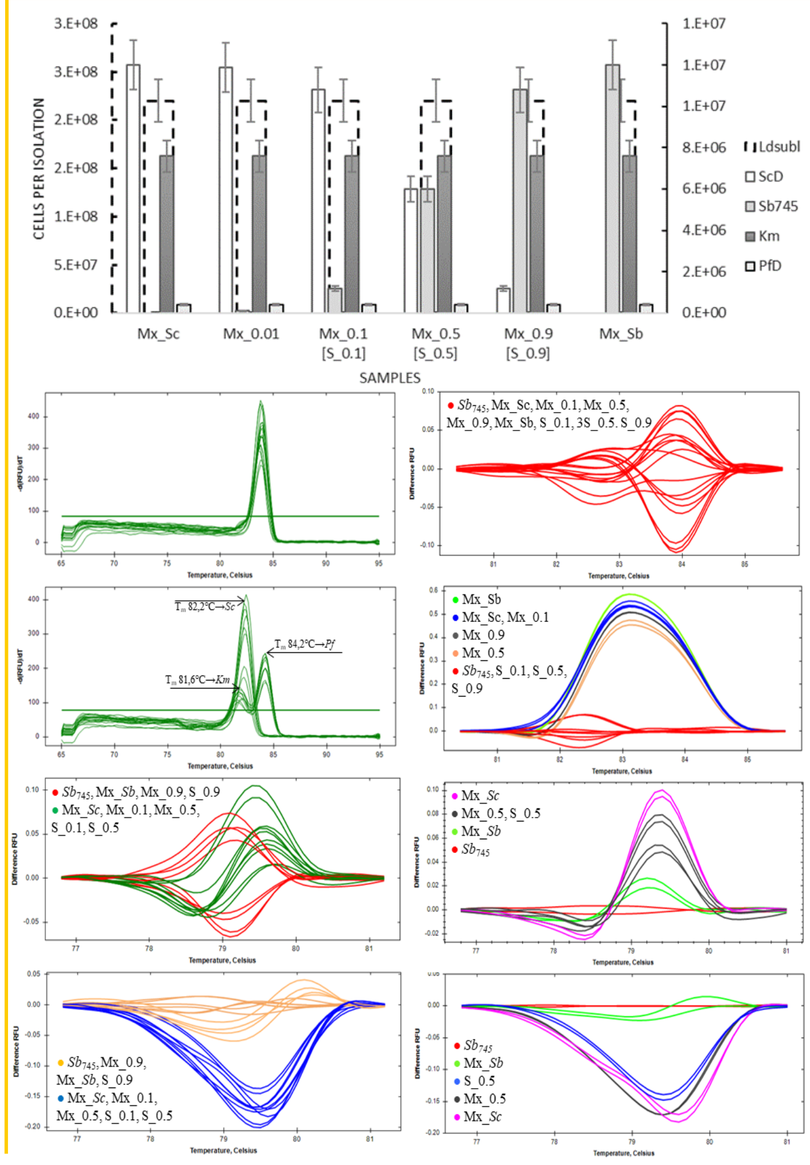
SACCHAROMYCES CEREVISIAE IDENTIFICATION

***Sb*$_{745}$** – reference strain *S. cerevisiae* var. *boulardii* CNCM I-745, ***Sc*$_{ATCC9763}$** – *Saccharomyces cerevisiae* 9763 from ATCC collection

SACCHAROMYCES CEREVISIAE VAR. *BOULARDII* IDENTIFICATION

POSITIVE *MAL11* AND *HXT9* AMPLIFICATION AND SPORULATION OF PS1 ISOLATE PROVED *SACCHAROMYCES CEREVISIAE* CONTENT

MULTI-YEAST MIXES CORRESPONDING TO MICROBIAL KEFIR COMPOSITION



MICROBIAL MIXTURES' QUANTITATIVE COMPOSITION

Ld*subl** - *Lactobacillus delbrueckii* subsp. *lactis* DSM 20072, ***Sc*$_D$** – isolate *S. cerevisiae* from DBFM collection, ***Km – *K. marxianus* DSM 5422, ***Pf*$_D$** – food-derived isolate *P. fermentans* from DBFM collection

18SrRNA AMPLICONS WERE GROUPED INTO ONE CLUSTER (R) – ΔT_M < 0,2°C (L)

ITS GROUPING DETERMINED BY MULTI-YEAST DNA TEMPLATE (R) – ΔT_M > 0,2°C (L)

HO AND ***RPB2*** AMPLICONS OF MIXTURES WITH 7 CFU ML^{-1} *Sb*$_{745}$ WERE CLUSTERED WITH REFERENCE (L)

The lower the proportion of *Sb*$_{745}$ cells in the suspension, the more the differential melting curve deviated from the reference red curve, shown in detailed graphs (R)

CONCLUSIONS

1. qPCR-HRM analysis with selected primer pairs is a rapid and effective tool for *Saccharomyces cerevisiae* var. *boulardii* identification in uniform yeast preparations.
2. Limited differentiation capacity of qPCR-HRM using designed interspecies primer pairs for rDNA regions in multi-yeast matrix was demonstrated.
3. High differentiation power of qPCR-HRM using *RPB2* selected sequence in multi-yeast matrix was concluded.
4. The predominant presence of *Saccharomyces cerevisiae* var. *boulardii* in a studied matrix is essential for qPCR-HRM identification.

The results presented are part of the manuscript: https://www.preprints.org/manuscript/202409.0492

Funding: subvention from the PULS fund (number 506.771.03.00) received from the Ministry of Science and Higher Education.