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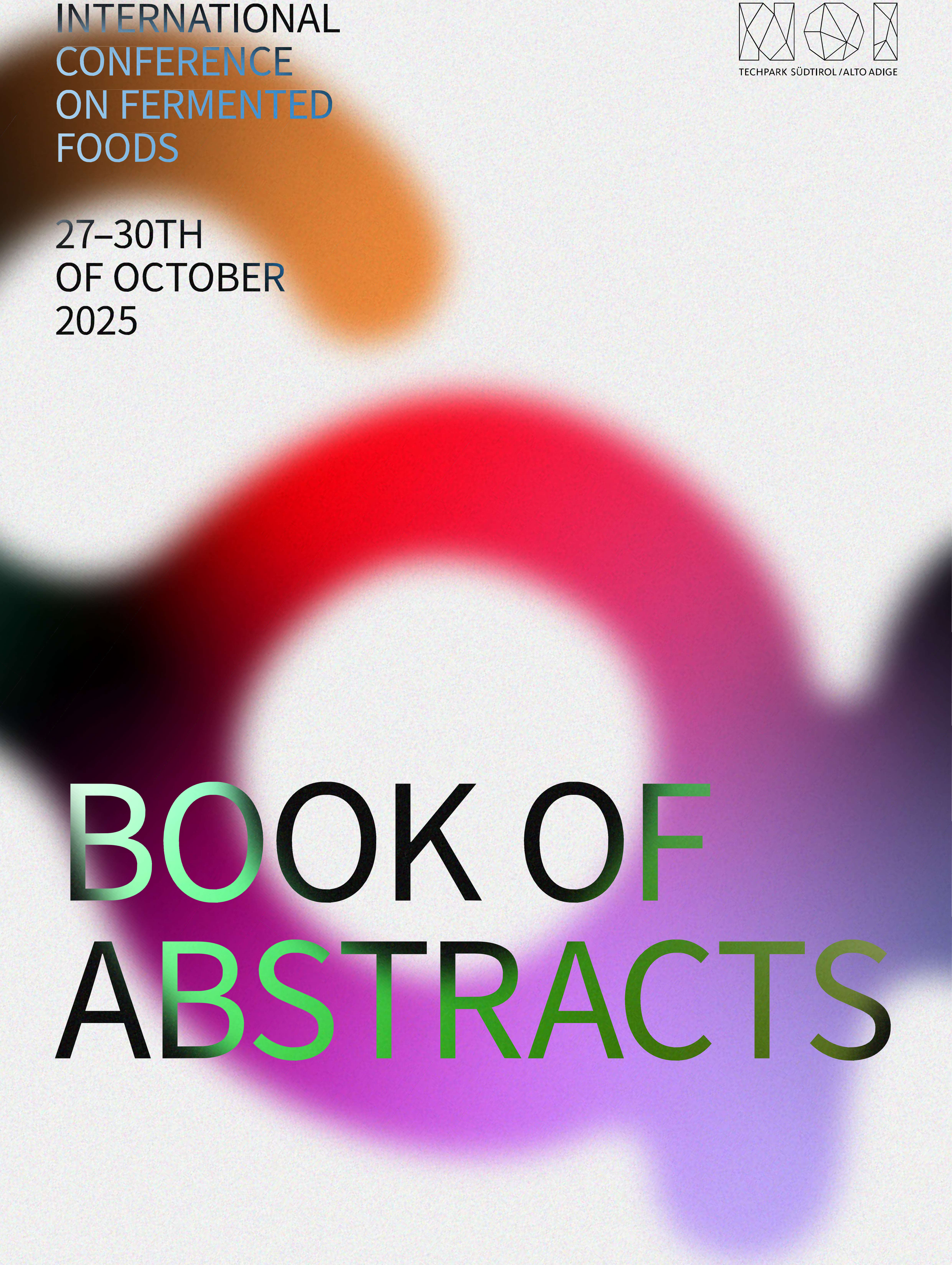

BOOK OF ABSTRACTS

Has *Lactobacillus helveticus* changed over 50 years? A genomic and technological characterization of recent and old strains.

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Lactobacillus helveticus (LH) is a thermophilic lactic acid bacterium commonly found in natural whey starter (NWS) cultures used for PDO cheese production, such as Parmigiano Reggiano. This study compares LH strains isolated 50 years ago and never revitalized with newly isolated strains from the same niche. After a preliminary genotypic and phenotypic screening, 16 old and 18 new strains were selected for whole-genome sequencing. The results indicate genomic decay over time: older strains exhibited significantly larger genomes (2.09 ± 0.17 Mbp) and higher GC content (37.52% ± 1.19) compared to recent ones (1.95 ± 0.03 Mbp; GC 36.76% ± 0.26). Phenotypic and techno-functional analyses further revealed diversity in dairy-related traits.

Four strains, two old (UNIBO 404, UNIBO 467) and two new (UPCCO 6358, UPCCO 6363), were selected for pilot-scale cheese production, co-inoculated with *Lactobacillus delbrueckii* subsp. *lactis* UPCCO 5365 to mimic traditional NWS cultures. Cheeses (aprox 1 kg) were manufactured in biological duplicates using pasteurized milk and following n experimental protocolsimulating cooked hard cheese production. The samples were ripened for three weeks at 16°C. Microbial dynamics were monitored via plate counts and qPCR and volatile compound profiles were compared through SPME-GC-MS. Findings highlight functional and metabolic differences in terms of microbial development and volatile compound production between the old and new LH strains, shedding light on microbial evolution within dairy fermentation ecosystems.

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