Traditional fresh sheep cheese
Karakačanski skakutanac

Research activities and outcomes
2007 – 2010

Tomislav Pogačić, PhD student
Karakačanski skakutanac

- Project: Functional dairy products made of sheep milk (Project No. 178-1782128-2124).

- Head of project: Prof.dr.sc. Dubravka Samaržija

- PhD student: Tomislav Pogačić

- Institution: University of Zagreb, Faculty of Agriculture Department of Dairy Science
Microbiological analysis
CATC, Rogosa, M17
Microbial consortia preparation
Bulk DNA isolation
Culture-dependent

Bulk DNA isolation
Culture-independent

CD&CI
PCR-ARDRA
PCR-DGGE
Sequencing of 16S rRNA gene

Casein particles isolation
Dynamic Light Scattering analysis

Chemical composition
Microbiological analysis
CATC, Rogosa, M17
Microbial consortia preparation
Bulk DNA isolation
Culture-dependent

Bulk DNA isolation
Culture-independent

Microbiological analysis
CD&CI
PCR-ARDRA
PCR-DGGE
Sequencing of 16S rRNA gene
1 ml from every plate ~ 5 ml

2 ml for DNA isolation

5 ml of consortia

3 ml for consortia preservation

5 plates / medium

Microbial consortia
Rogosa, CATC, M17 - 5 plates/medium/consortia
33 cheeses → 99 consortia / 3 ml
20 aliquotes of every consortia / 100 ul

Storage – at -20°C

Glycerol 30% v/v

aliquotes – 20/consortia
MOLECULAR CHARACTERIZATION OF THE MICROBIAL COMMUNITY ASSOCIATED WITH TRADITIONAL FRESH SHEEP CHEESE KARAKAČANSKI SKAKUTANAC

DNA extraction from cheeses
33 DNA isolates (Maxwell system – Promega)

Consortium (BULK COLONY) Rogosa – 33 samples (10^-2)
Consortium (BULK COLONY) CATC – 33 samples (10^-3)
Consortium (BULK COLONY) M17 – 33 samples (10^-7)

DNA extraction from bacterial consortium
99 DNA isolates

PCR (16S rDNA)
PA ; PH primers
ARDRA - TRU 1I enzyme

PCR (V3-16S rDNA)
V3r, V3fGC primers

Sequencing of PCR product in case of single species profile

DGGE
Sequencing of bands on different position

PCR (16S rDNA)
V3r, V3fGC primers

ARDRA - TRU 1I enzyme

PCR (V3-16S rDNA)
V3r, V3fGC primers

DGGE
Sequencing of bands on different position

Sequencing of PCR product in case of single species profile


Further steps

- Genetic and functional characterization of indigenous isolates
- Single strain – mix strains functionality assessment
- ...
PhD research training
Slovenia
University of Ljubljana
Biotechnical Faculty
Department of Animal Science
Chair of Dairy Science

Culturing; consortia preparation;
DNA isolation
March/April 2008
Italy

Universita di Padova
Facolta di Agraria
Campus di Conegliano
CIRVE – Laboratorio di microbiologia

PCR-ARDRA; PCR for DGGE
May – July – 2008
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Research interests:
- Microbial ecology of raw milk cheeses
- Indigenous LAB – genetic and functional characterization
- Cheese metagenomic

Publications - Web of Science
Publications – CROSBI
“There is no room for competition, there is just room for collaboration. The task is so huge.”