

D9 - Molecular characterisation of the indigenous microflora of artisanal fresh sheep cheese Karakačanski skakutanac by PCR-ARDRA & PCR-DGGE

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Artisanal fresh sheep cheese Karakačanski skakutanac is a small scale produced cheese typical of the limited area of eastern Croatia. The traditional technology of its manufacture is focused on the use of unrefrigerated raw sheep milk immediately after milking, without addition of starter culture and curding is achieved using industrial rennet. No information related to microbiological or molecular characterization of its indigenous microbiota has been performed. The purpose of the present study was to give a first insight into the indigenous microbiota associated with this cheese. Samples of cheeses (n=11) were collected from a dairy farm every 14 days during lactation season of east Friesian sheep, in 2007. Modified Maxwell 16 System protocol was used for DNA extraction directly from cheese samples collected on the first, second and third day of cheese shelf

life, resulting in a total of 33 samples. Extracted DNA was used as a template in PCR-ARDRA and PCR-DGGE analysis. ARDRA profiles of the species revealed the presence of a single species, *Lactococcus lactis* subsp. *lactis*, and not a mixed microflora which would be expected due to the fact that the cheese is made of raw milk. The DGGE method, showed only one dominant band on the same position for all samples which after sequencing analysis showed 100% identity with *Lactococcus lactis* subsp. *cremoris*. The results of both method were concomitant and indicated absolute dominance of *Lactococcus lactis* in this cheese. The fact that no shift in structure of microbial community was observed remains still to be elucidated. Further genetic and functional characterization of the microbiota is in progress.

Keywords: Karakačanski skakutanac, ARDRA, DGGE, *Lactococcus lactis*