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Enumeration, isolation and identifi cation of lactic acid bacteria from the Italian traditional fermented milk "Gioddu"

Modesto M.1, Carminati D.2, Luiselli D.3, Corona A.3, Sciavilla P.1, Mattarelli P.1, Giraffa G.2

¹University of Bologna, Department of Agricultural and Food Science, Bologna, Italy, ²Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria (CREA), Centro di Ricerca Zootecnia e Acquacoltura (CREA-ZA), Lodi, Italy, ³University of Bologna, Department of Biological, Geological and Environmental Sciences, Bologna, Italy

In many areas of the world traditional dairy products rely on spontaneous milk fermentations which result in a large variety of products with different flavours and containing important beneficial microorganisms. Gioddu, a traditional fermented milk, is prepared in the very restricted areas of north Sardinia (Italy) from sheep or goat milk and it is a typical meal of shepherds. Gioddu is prepared as follows: the row milk is added with calf rennet and maintained firstly for 30 min at 30-40°C. then for one-two days at room temperature. This preparation is used as natural starter culture ("sa madrighe") and added at a rate of 1-2% to fresh milk previously boiled for 5 min and cooled to about 30-40 °C. After 8 hours the Gioddu is ready. Gioddu can be obtained also after adding boiled milk with the previous day's Gioddu production. The objective of this study was to investigate the diversity of lactic acid bacteria (LAB) communities in 5 different samples of Gioddu and madrighe collected from 3 different areas in Sardinia, with culture dependent and independent techniques.

The LAB counts in these samples varied from 10⁵ and 10⁵ ufc/ml. Fermented milks had almost identical mean numbers of LAB. In total, 100 isolates were obtained from these samples using MRS agar and M17 agar incubated at 25, 37 and 42°C in order to widen the possibility to isolate different strains. Each isolate was considered to be presumptive LAB based on gram-positive and catalase-negative properties and on RAPD-PCR. Basing on RAPD-PCR database the following species have been presumptively identified *Lactococcus lactis, Streptococcus thermophilus, Leuconostoc mesenteroides, Enterococcus* spp. and *Lactobacillus paracasei*. LH-PCR fingerprinting, associated to the sequencing of 16S rRNA genes, was used for the characterization of the bacterial community and to confirm identification of species in the 5 Gioddu and Gioddu starters tested. The resulting LAB confirmed the results of RAPD-PCR with two main LAB associations: in 2 samples the prevalent species were *Lactococcus/Streptococcus, Leuconostoc, Enterococcus* while in the other 3 samples *Lactococcus, Streptococcus, Leuconostoc*. These results can help to understand more about Gioddu which is a traditional fermented product until now poorly investigated. Due to the importance of finding new probiotics, further studies will include challenges from a phylogenetic point of view and for screening the functional properties of the strains isolated.

Keywords: Gioddu, lactic acid bacteria, fermented milk, Sardinia