



Project SUS-MIRRI.IT

“Strengthening the MIRRI Italian Research Infrastructure for Sustainable Bioscience and Bioeconomy”
Area ESFR “Health and Food”, granted by the European Commission – NextGenerationEU
Code N° IR0000005

BANDO N. 400.5 ISA PNRR

Selezione per titoli e colloquio ai sensi dell'art. 8 del "Disciplinare concernente le assunzioni di personale con contratto di lavoro a tempo determinato", per l'assunzione, ai sensi dell'art. 83 del CCNL del Comparto "Istruzione e Ricerca" 2016-2018, sottoscritto in data 19 aprile 2018, di una unità di personale con profilo professionale di Collaboratore tecnico enti di ricerca - VI livello, presso l'Istituto di Scienze dell'Alimentazione del CNR - sede Avellino - Progetto PNRR - “Strengthening the MIRRI Italian Research Infrastructure for Sustainable Bioscience and Bioeconomy” (acronimo: SUS-MIRRI.IT) – cod. IR0000005 - CUP D13C22001390001.

DOMANDE PREDISPOSTE DALLA COMMISSIONE

BUSTA N.1 (ESTRATTA)

- 1) Il candidato descriva le principali tecniche di identificazione dei microrganismi
- 2) Il candidato descriva i principali metodi di conservazione dei microrganismi
- 3) L'accertamento della conoscenza della lingua inglese sarà effettuato attraverso la lettura e traduzione dell'abstract dell'articolo di seguito riportato:



microorganisms



Article

Isolation and Characterization of Lactic Acid Bacteria and Yeasts from Typical Bulgarian Sourdoughs

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Abstract: Traditional sourdoughs in Bulgaria were almost extinct during the centralized food production system. However, a rapidly developing trend of sourdough revival in the country is setting the demand for increased production and use of commercial starter cultures. The selection of strains for such cultures is based on geographical specificity and beneficial technological properties. In this connection, the aim of this study was to isolate, identify and characterize lactic acid bacteria (LAB) and yeasts from typical Bulgarian sourdoughs for the selection of strains for commercial sourdough starter cultures. Twelve samples of typical Bulgarian sourdoughs were collected from different geographical locations. All samples were analyzed for pH, total titratable acidity and dry matter content. Enumeration of LAB and yeast was also carried out. Molecular identification by 16S rDNA sequence analysis was performed for 167 LAB isolates, and 106 yeast strains were identified by ITS1-5.8S-ITS2 rRNA gene partial sequence analysis. The LAB strains were characterized according to their amylolytic and proteolytic activity and acidification capacity, and 11 strains were selected for further testing of their antimicrobial properties. The strains with the most pronounced antibacterial and antifungal activity are listed as recommended candidates for the development of starter cultures for sourdoughs or other food products.

Keywords: Bulgarian sourdoughs; lactic acid bacteria; yeast; microbial characterization



Citation: Petkova, M.; Stefanova, P.; Gotcheva, V.; Angelov, A. Isolation and Characterization of Lactic Acid Bacteria and Yeasts from Typical Bulgarian Sourdoughs. *Microorganisms* **2022**, *9*, 1346. <https://doi.org/10.3390/microorganisms9071346>

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BUSTA N.2

- 1) Il candidato descriva i metodi di coltivazione dei lieviti
- 2) Il candidato descriva come preparare un vetrino per l'osservazione al microscopio di microrganismi
- 3) L'accertamento della conoscenza della lingua inglese sarà effettuato attraverso la lettura e traduzione dell'abstract dell'articolo di seguito riportato:



ORIGINAL RESEARCH
published: 02 February 2021
doi: 10.3389/fmicb.2021.628100



Isolation, Identification and Characterization of Yeasts from Fermented Goat Milk of the Yaghnob Valley in Tajikistan

Linnea A. Qvirist¹, Carlotta De Filippo², Francesco Strati³, Irene Stefanini⁴, Maddalena Sordo⁵, Thomas Andlid⁶, Giovanna E. Feis⁴, Paola Mattarelli⁷ and Duccio Cavalieri^{1*}

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The geographically isolated region of the Yaghnob Valley, Tajikistan, has allowed its inhabitants to maintain a unique culture and lifestyle. Their fermented goat milk constitutes one of the staple foods for the Yaghnob population, and is produced by back-slopping, i.e., using the previous fermentation batch to inoculate the new one. This study addresses the yeast composition of the fermented milk, assessing genotypic and phenotypic properties. The 52 isolates included in this study revealed small species diversity, belonging to *Kluyveromyces marxianus*, *Pichia fermentans*, *Saccharomyces cerevisiae*, and one *Kazachistania unispora*. The *K. marxianus* strains showed two different genotypes, one of which never described previously. The two genetically different groups also differed significantly in several phenotypic characteristics, such as tolerance toward high temperatures, low pH, and presence of acid. Microsatellite analysis of the *S. cerevisiae* strains from this study, compared to 350 previously described strains, attributed the Yaghnobi *S. cerevisiae* to two different ancestry origins, both distinct from the wine and beer strains, and similar to strains isolated from human and insects feces, suggesting a peculiar origin of these strains, and the existence of a gut reservoir for *S. cerevisiae*. Our work constitutes a foundation for strain selection for future applications as starter cultures in food fermentations. This work is the first ever on yeast diversity from fermented milk of the previously unexplored area of the Yaghnob Valley.

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Specialty section:

This article was submitted to
Food Microbiology,
a section of the journal

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Area ESFRI “Health and Food”, granted by the European Commission – NextGenerationEU
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BUSTA N.3 (ESTRATTA)

- 1) Il candidato descriva le caratteristiche di una biobanca microbica
- 2) Il candidato descriva la tecnica di colorazione di GRAM
- 3) L'accertamento della conoscenza della lingua inglese sarà effettuato attraverso la lettura e traduzione dell'abstract dell'articolo di seguito riportato:



ORIGINAL RESEARCH
published: 24 July 2019
doi: 10.3389/fmicb.2019.01867



Lactic Acid Bacteria Biota and Aroma Profile of Italian Traditional Sourdoughs From the Irpinian Area in Italy

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Edited by:
Risako TAJIMA,
University of Tsukuba, Japan

Reviewed by:
Lucia Salvadori,
Centro de Referencia para
Lactobacilos (CRESLA)-CONICET,
Argentina
Giorgio Gratta,
Research Centre for Animal
Production and Aquaculture (CREA),
Italy

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www.frontiersin.org

Specialty section:
This article was submitted to
Food Microbiology,
a section of the journal
Frontiers in Microbiology

Received: 03 April 2019
Accepted: 01 July 2019
Published: 24 July 2019

Citation:
Reale A, Di Renzo T, Boscalino F,
Nazzaro F, Fratiani F and Aponte M
(2019) Lactic Acid Bacteria Biota and
Aroma Profile of Italian Traditional
Sourdoughs From the Irpinian Area in

This study identified the lactic acid bacteria (LAB) biota and the volatile profile of 28 typical sourdoughs of Irpinia—a large area of the Campania region of Southern Italy where numerous breads are produced, even today, following the ancient procedures of sourdough fermentation and for which information on the microbiological and sensory profile is lacking in literature. For this purpose, microbial quality, LAB biodiversity, chemical, and technological characteristics, as well as aroma profile by solid-phase microextraction technique (SPME)—gas chromatography/mass spectrometry (GC/MS) of Irpinian sourdoughs were investigated. The dominant LAB microbiota was examined by both culture-dependent and culture-independent methods. Polymerase Chain Reaction/Denaturing Gradient Gel Electrophoresis (PCR-DGGE). Results showed a high biodiversity in LAB community whereas the most frequent lactobacilli species recognized were *Lactobacillus plantarum* (ca. 22% of total LAB isolates), *Lactobacillus sanfranciscensis* (11%), *Lactobacillus paralimentarius* (8%), and *Lactobacillus rossiae* (6.5%), whereas LAB cocci could be mainly referred to *Pedococcus pentosaceus* (9.5% of total LAB isolates), *Leuconostoc* spp. (7.8%), and *Weissella cibaria* (7.7%). Sourdoughs were characterized by the dominance of one or two LAB species, thus proving that the environment influences the selection and the establishment of few key LAB species and that no specific correlation can be traced between microbial composition and geographical origin of the samples. Furthermore, although sourdoughs were characterized by different qualitative and quantitative volatile organic compound (VOC) compositions, no noticeable correlation between volatile profile and geographical origin was found. However, it emerged that for more isolated locations, it was possible to find the existence of microbial biotypes and sensory profiles with a strong identity, thus revealing the existence of highly traditional and evocative bread recipes in those geographical contexts.

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BUSTA N.4

- 1) Il candidato descriva le principali caratteristiche dei lieviti
- 2) Il candidato descriva le principali tecniche di identificazione genetica dei microrganismi
- 3) L'accertamento della conoscenza della lingua inglese sarà effettuato attraverso la lettura e traduzione dell'abstract dell'articolo di seguito riportato:

Brazilian Journal of Microbiology (2021) 52:869–881
<https://doi.org/10.1007/s42770-021-00461-y>



FOOD MICROBIOLOGY - RESEARCH PAPER



Identification and characterisation of the lactic acid bacteria associated with the traditional fermentation of dairy fermented product

Promiselynda Ijeoma Obioha¹ · L. Irene I. Ouoba^{1,2} · Amarachukwu Anyogu³ · Brigitte Awamaria¹ · Sarah Atchia¹ · Philippa C. Ojimekwe⁴ · Jane P. Sutherland¹ · Hamid B. Ghoddsi¹

Received: 13 July 2020 / Accepted: 23 February 2021 / Published online: 11 March 2021
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Abstract

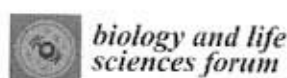
The aim of this research was to identify the key lactic acid bacteria associated with the fermentation of dairy traditional fermented products for developing starter cultures for controlled fermentation. A total of 100 lactic acid bacteria (LAB) were isolated from dairy traditional fermented products. Samples were obtained from eight producers in the South East of Nigeria. Isolates were identified by phenotypic and genotypic techniques including rep-PCR genotyping and sequencing of the 16S rRNA, pheS and rpoA genes. Isolates were characterised for antimicrobial activity against foodborne pathogens, exopolysaccharide (EPS) production and survival at low pH and in the presence of bile salts. All isolates clustered into 11 distinct rep-PCR groups and were identified as *Lactobacillus fermentum* (40%), *Lactobacillus delbrueckii* (23%), *Streptococcus thermophilus* (22%), *Streptococcus infantarius* (10%), *Lactobacillus senioris* (2%), *Leuconostoc pseudomesenteroides* (2%) and *Enterococcus thailandicus* (1%). *Lactobacillus fermentum* showed a broad spectrum antimicrobial activity and survival at low pH, while *Lactobacillus delbrueckii* was able to tolerate low pH and produce EPS. All isolates survived in vitro exposure to 1% (w/v) bile salts over a 3-h period. *L. fermentum*, *L. delbrueckii* and *S. thermophilus* could be used to simulate the fermentation of dairy traditional fermented products.

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BUSTA N.5 (ESTRATTA)

- 1) Il candidato descriva le attività di isolamento e purificazione di un microorganismo
- 2) Il candidato descriva le principali caratteristiche dei batteri lattici
- 3) L'accertamento della conoscenza della lingua inglese sarà effettuato attraverso la lettura e traduzione dell'abstract dell'articolo di seguito riportato:



Proceeding Paper

Detection and Identification of Lactic Acid Bacteria in Semi-Finished Beer Products Using Molecular Techniques †

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† Presented at the 2nd International Electronic Conference on Foods—Future Foods and Food Technologies for a Sustainable World, 15–30 October 2021; Available online: <https://food-2021.sciforum.net/>.



Citation: Tsekouras, G.; Tryfinopoulou, P.; Panagou, E.Z. Detection and Identification of Lactic Acid Bacteria in Semi-Finished Beer Products Using Molecular Techniques. *Biol. Life Sci. Forum* **2022**, *4*, 535. <https://doi.org/10.3390/bls2022000535>

Abstract: Beer has been generally recognized as a microbiologically stable beverage. However, deviations in the brewing process may occur due to the activity of lactic acid bacteria (LAB). The growth of LAB during the brewing process implies a competition for nutrients with yeasts, causing decreased ethanol yields. Moreover, quality degradation caused by LAB spoilage can be observed due to the production of off-flavors (high indications of diacetyl and lactic acid), changes in color and excessive turbidity. This study aims at the microbiological investigation of non-pasteurized beer products, before and after filtration, with the main emphasis on the detection and molecular characterization of the biodiversity of LAB. Sampling was performed at selected points in a beer production line on the industrial scale in order to determine the population of Total Viable Counts (TVC), yeasts and LAB. The samples are classified in the "lager" category, fermented using strains of *Saccharomyces pastorianus*. The sampling points included the pre- and post-filtration step, the buffer line, the filling tank, the packaged but non-pasteurized product and finally, the packaged pasteurized product to confirm the effectiveness of heat treatment. Samples were collected in two different batch productions. The results showed that the population of LAB was relatively low. Specifically, before filtration, levels were 1.52 log CFU/mL and 3.44 log CFU/mL in the first and second batch, respectively. This microbial group was not enumerated (<1.0 log CFU/mL) afterwards in all sampling points. A total of 80 LAB species were initially analyzed by rep-PCR, using the (GTG)₅ primer to discriminate the isolates. Representative isolates (20) were selected for further identification using the conserved 16S rRNA region to be sequenced. Three different species were present in both batch productions, namely *Lactobacillus brevis*, *Lactobacillus backii* and *Lactobacillus turbidus*.

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BUSTA N.6

- 1) Il candidato descriva i metodi di coltivazione dei batteri lattici
- 2) Il candidato descriva una tecnica per valutare l'attività antimicrobica di un microrganismo
- 3) L'accertamento della conoscenza della lingua inglese sarà effettuato attraverso la lettura e traduzione dell'abstract dell'articolo di seguito riportato:



bacteria



Article

Isolation and Identification of Autochthonous Lactic Acid Bacteria from Commonly Consumed African Indigenous Leafy Vegetables in Kenya

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Abstract: African indigenous leafy vegetables (AILVs) are plants that have been part of the food systems in Sub-Saharan Africa (SSA) for a long time and their leaves, young shoots, flowers, fruits and seeds, stems, tubers, and roots are consumed. These vegetables are high in vitamins, minerals, protein, and secondary metabolites that promote health. This study aimed at isolating, characterizing, and identifying dominant lactic acid bacteria (LAB) from naturally fermenting commonly consumed AILV in Kenya. A total of 57 LAB strains were isolated and identified based on phenotypic and 16S rRNA gene analyses from three AILVs (23 nightshade leaves, 19 cowpea leaves, and 15 vegetable amaranth). The highest microbial counts were recorded between 48 h and 96 h of fermentation in all AILVs ranging from approximately log 8 to log 9 CFU/mL with an average pH of 3.7. Fermentation of AILVs was dominated by twenty eight *Lactobacillus* spp. [*Lactiplantibacillus plantarum* (22), *Limosilactobacillus fermentum* (3), *Lactiplantibacillus pentosus* (2) and *Lactiplantibacillus casei* (1)], eleven *Weissella* spp. [*Weissella cibaria* (8), *W. confusa* (2), and *W. muntiaci*] six *Leuconostoc* spp. [*Leuconostoc mesenteroides* (3), *Leuc. citreum* (2) and *Leuc. lactis* (1)], six *Pediococcus pentosaceus*, four *Enterococcus* spp. [*Enterococcus mundtii* (2), *E. faecalis* (1) and *E. durans* (1)] and, finally, two *Lactococcus garvicus*. These bacteria strains are commonly used in food fermentation as starter cultures and as potential probiotics.



Citation: Wafula, E.N.; Kuja, J.O.; Wekesa, T.B.; Wanjala, P.M. Isolation and Identification of Autochthonous

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