



## Project SUS-MIRRI.IT

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

### BANDO N. 400.6 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 N1

- 1) Il candidato descriva le tecniche di isolamento, conservazione e gestione di un microorganismo in una biobanca microbica.
- 2) Il candidato descriva uno strumento informatico per realizzare una presentazione.

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ORIGINAL PAPER



### Identification and in vitro evaluation of probiotic attributes of lactic acid bacteria isolated from fermented food sources

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#### Abstract

Consumer's vigilance towards health-promoting foods beyond only taste and nutrition has increased the recognition for probiotic products. In the present study, various parameters have been studied to define the probiotic properties of cultures isolated from different fermented products. Around 118 samples were selectively screened for antimicrobial compound (AMC) producing isolates by overlay-plate assay using *Micrococcus luteus* ATCC9341. Among 134 zone producing isolates, 48 cultures showing Gram-positive, catalase negative, non-spore forming and non-motile rods and cocci were selected. Subsequently, 18 strains were chosen based on non-hemolytic, absence of biogenic amine production, gelatinase and lecithinase negative trait for safer isolates. These were identified by biochemical assays and then subjected to RAPD-PCR. The selected cultures DB-1aa, DB-b2-15b, Cu2-PM7, Cu3-PM8 and IB-pM15 were identified by 16S rDNA sequencing as *Enterococcus durans*, *Enterococcus faecium*, *Lactobacillus plantarum*, and two *Lactobacillus fermentum*, respectively. Several in vitro experiments were carried out including acid and bile tolerance, survival under simulated gastrointestinal condition, adhesion assay to evaluate the probiotic potential of the isolates. In addition, the isolates were studied for competent properties such as antibacterial, antioxidant activity, and enzyme production for their functional application. The results of the study prove the efficiency of selected isolates as potential probiotic cultures and hence can be recommended for application in any functional food formulations.



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### BUSTA N2

- 1) Il candidato descriva in breve le fasi di estrazione, amplificazione e sequenziamento del dna microbico
- 2) Il candidato descriva uno strumento informatico per raggruppare un insieme di dati omogenei.



fermentation



Article

# Probiotic Properties of Lactic Acid Bacteria Isolated from the Spontaneously Fermented Soybean Foods of the Eastern Himalayas

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**Abstract:** Spontaneously fermented soybean foods with sticky-textured and umami-flavor are popular delicacies of multi-ethnic communities of the Eastern Himalayas. Even though species of *Bacillus* have been reported earlier as pre-dominant bacteria, we hypothesized that some lactic acid bacteria (LAB) present in these unique soy-based foods may exhibit probiotic properties. Hence, the present study is aimed to evaluate some probiotic attributes of LAB. A total of 352 bacterial isolates from spontaneously fermented soybean foods of the Eastern Himalayas viz., *kinema*, *grep-chhurpi*, *peha*, *peron namasing* and *peruñyaan* were preliminarily screened for survival in low pH, bile salt tolerance, and cell surface hydrophobicity. Finally, eight probiotic LAB were selected and identified, based on the 16S rRNA gene sequencing, as *Pediococcus acidilactici* Ki20 and *Enterococcus faecium* Kn19 (isolated from *kinema*), *E. faecalis* Gc21 (*grep-chhurpi*), *P. acidilactici* Ph32 (*peha*), *E. faecium* Pn11 and *E. faecalis* Pn37 (*peron namasing*), *E. lactis* Py03, and *E. lactis* Py08 (*peruñyaan*). In vitro probiotic attributes, *E. faecium* Kn19 ( $73.67 \pm 1.05$ ) and *P. acidilactici* Ph32 ( $79.71 \pm 0.13\%$ ) recorded higher survival ability in acid and bile salt test, respectively. Furthermore, attachment ability of isolates to hydrocarbons showed  $\geq 80\%$  adhesion property with *E. faecalis* Gc21 ( $90.50 \pm 10.14\%$ ) marked the highest degree of hydrophobicity, and *P. acidilactici* Ki20 showed the higher auto-aggregation and co-aggregation property. LAB strains were able to produce antibacterial activity against pathogenic bacteria. Genetic screening revealed the presence of genes responsible for acid tolerance (*groEl*, *clpL*), bile salt tolerance (*apf*, *bsh*), adhesion (*msa*, *mub1*), and bacteriocin producing genes for pediocin (*pedA*, *pedB*) and enterocin (*entA*, *entB*). The present study highlighted the probiotic potentials of LAB strains isolated from Himalayan naturally fermented soybean foods that may be developed as a starter or co-starter culture for controlled and optimized fermentation of soybeans.

**Keywords:** lactic acid bacteria; probiotic; *kinema*; spontaneously fermented soybeans; *Pediococcus*; *Enterococcus*



**Citation:** Kharnaier, P.; Tamang, J.P. Probiotic Properties of Lactic Acid Bacteria Isolated from the Spontaneously Fermented Soybean Foods of the Eastern Himalayas. *Fermentation* **2023**, *9*, 461. <https://doi.org/10.3390/fermentation9050461>



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### BUSTA N3

- 1) Il candidato descriva una tecnica per valutare l'attività antimicrobica di un microorganismo
- 2) Il candidato descriva come applicare una formula su un foglio elettronico (tipo MS Excell)



microorganisms



Article

## Exploring the Health Benefits of Yeast Isolated from Traditional Fermented Foods in Korea: Anti-Inflammatory and Functional Properties of *Saccharomyces* and Non-*Saccharomyces* Strains

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**Abstract:** Traditional yeast (*Saccharomyces cerevisiae*) has been used for its benefits in various fermentation processes; the benefits of non-*Saccharomyces* yeast as a material for food, feed, and pharmaceuticals have been studied recently. This study evaluated the anti-inflammatory activity and extracellular functional characteristics of wild-type yeasts isolated from traditional fermented foods (*doenjang* (common name: soybean paste) and *nuruk*) in Korea. The viability of the yeast and lipopolysaccharide (LPS)-stimulated RAWBlue™ cells was improved, similar to unstimulated RAWBlue™ cells, and the isolates demonstrated NF-κB inhibitory activity. Yeast suppressed the nitric oxide production in LPS-stimulated RAWBlue™ cells, which was attributed to the inhibition of iNOS or COX-2 mRNA expression depending on the strain. Although there were differences depending on the strain, the production of anti-inflammatory cytokines was reduced in the yeast and LPS-stimulated RAWBlue™ cells, some of which were demonstrated at the mRNA level. In addition, the isolates exhibited high antioxidant and antihypertensive activities (similar to the positive control), which varied depending on the strain. This suggests that yeast can be used for fermentation with enhanced antioxidant and antihypertensive activities. Furthermore, the isolates inhibited the growth of pathogenic Gram-negative bacteria, indicating that yeast can inhibit food spoilage and the growth of pathogenic bacteria during fermentation. Consequently, utilizing raw materials to cultivate yeast strains could be a promising avenue for developing functional foods to prevent and treat inflammatory reactions; such foods may exhibit antioxidant, antihypertensive, and antibacterial properties.

**Keywords:** *Saccharomyces*; non-*Saccharomyces*; anti-inflammatory activity; functional property



**Citation:** Jeong, W.-S.; Kong, H.-R.; Kim, S.-Y.; Yeo, S.-H. Exploring the Health Benefits of Yeast Isolated from Traditional Fermented Foods in Korea: Anti-Inflammatory and Functional Properties of *Saccharomyces* and Non-*Saccharomyces* Strains. *Microorganisms* **2023**, *11*, 1503. <https://doi.org/10.3390/microorganisms11061503>



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### BUSTA N4 (ESTRATTA)

- 1) Il candidato descriva le principali tecniche di identificazione genetica dei microrganismi
- 2) Il candidato descriva quali pacchetti software conosce.



fermentation



Article

## Biodiversity of Lactic Acid Bacteria in Traditional Fermented Foods in Yunnan Province, China, and Comparative Genomics of *Lactobacillus plantarum*

Hong Li <sup>1,2,3</sup>, Jiang Zhu <sup>1,2,3</sup>, Yue Xiao <sup>1,2,3</sup>, Shiyao Zhang <sup>1,2,3</sup>, Yuwei Sun <sup>1,2,3</sup>, Zhijia Liu <sup>1,2,3</sup>, Chuanqi Chu <sup>4</sup>, Xiaosong Hu <sup>1,2,3,5</sup> and Junjie Yi <sup>1,2,3,\*</sup>

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**Abstract:** The diversity of lactic acid bacteria (LAB) in fermented foods in Yunnan currently lacks large-scale and systematic research. A total of 638 fermented foods were collected from 84 regions in Yunnan for diversity analyses. The results show that the dominant strains in various types of fermented foods were different. Additionally, the majority of the LAB were better adapted to regions with a temperature of 15–20 °C and a humidity of 64–74%. *Lactobacillus plantarum* (*L. plantarum*) was the most abundant of all the strains and was widely distributed in the 84 regions. Genetically, the guanine plus cytosine (GC) content of *L. plantarum* ranged from 35.60% to 47.90%, with genome sizes from 2.54 Mb to 5.76 Mb. A phylogenetic analysis revealed that the habitat source and geographic origin had little influence on the homologous genes of *L. plantarum*. The genetic diversity of *L. plantarum* was mostly represented by functional genes and carbohydrate utilization. This research provides valuable insights into the microbiota of different types of fermented foods in Yunnan. Meanwhile, a genetic diversity analysis of *L. plantarum* may help us to understand the evolutionary history of this species.

**Keywords:** biodiversity; lactic acid bacteria; fermented foods; comparative genomics; carbohydrate utilization



**Citation:** Li, H.; Zhu, J.; Xiao, Y.; Zhang, S.; Sun, Y.; Liu, Z.; Chu, C.; Hu, X.; Yi, J. Biodiversity of Lactic Acid Bacteria in Traditional Fermented Foods in Yunnan Province, China, and Comparative Genomics of *Lactobacillus plantarum*. *Fermentation* **2023**, *9*, 402. <https://>





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### BUSTA N5

- 1) Il candidato descriva come si prepara un substrato di coltura microbica e ne descriva le caratteristiche
- 2) Il candidato descriva cosa è un database relazionale.



# Physico-chemical and microbiological characterization of spontaneous fermentation of Cellina di Nardò and Leccino table olives

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Table olives are one of the most important traditional fermented vegetables in Europe and their world consumption is constantly increasing. In the Greek style, table olives are obtained by spontaneous fermentations, without any chemical debittering treatment. Evolution of sugars, organic acids, alcohols, mono, and polyphenol compounds and volatile compounds associated with the fermentative metabolism of yeasts and bacteria throughout the natural fermentation process of the two Italian olive cultivars Cellina di Nardò and Leccino were determined. A protocol was developed and applied aimed at the technological characterization of lactic acid bacteria (LAB) and yeast strains as possible candidate autochthonous starters for table olive fermentation from Cellina di Nardò and Leccino cultivars. The study of the main physico-chemical parameters and volatile compounds during fermentation helped to determine chemical descriptors that may be suitable for monitoring olive fermentation. In both the analyzed table olive cultivars, aldehydes proved to be closely related to the first stage of fermentation (30 days), while higher alcohols (2-methyl-1-propanol; 3-methyl-1-butanol), styrene, and o-cymene were associated with the middle stage of fermentation (90 days) and acetate esters with the final step of olive fermentation (180 days).

**Keywords:** table olives, yeast, lactic acid bacteria, volatile compounds, fermented food





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### BUSTA N6

- 1) Il candidato descriva le tecniche di conservazione di ceppi microbici
- 2) Il candidato descriva quali sono i principali strumenti di office automation



fermentation



Article

## Assessment of Starters of Lactic Acid Bacteria and Killer Yeasts: Selected Strains in Lab-Scale Fermentations of Table Olives (*Olea europaea* L.) cv. *Leccino*

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**Abstract:** Olives debittering, organoleptic quality and safety can be improved with yeasts and lactic acid bacteria (LABs) selected strain starters, that allow for better fermentation control with respect to natural fermentation. Two selected killer yeasts (*Wickerhamomyces anomalus* and *Saccharomyces cerevisiae*) and *Lactobacillus plantarum* strains were tested for olive (cv. *Leccino*) fermentation to compare different starter combinations and strategies; the aim was to assess their potential in avoiding pretreatments and the use of excessive salt in the brines and preservatives. Lactobacilli, yeasts, molds, *Enterobacteriaceae* and total aerobic bacteria were detected, as well as pH, soluble sugars, alcohols, organic acids, phenolic compounds, and rheological properties of olives. Sugars were rapidly consumed in the brines and olives; the pH dropped quickly, then rose until neutrality after six months. The oleuropein final levels in olives were unaffected by the treatments. The use of starters did not improve the LABs' growth nor prevent the growth of *Enterobacteriaceae* and molds. The growth of undesirable microorganisms could have been induced by the availability of selective carbon source such as mannitol, whose concentration in olive trees rise under drought stress. The possible role of climate change on the quality and safety of fermented foods should be furtherly investigated. The improvement of olives' nutraceutical value can be induced by yeasts and LABs starters due to the higher production of hydroxytyrosol.

**Keywords:** table olives; fermentation; starter cultures; killer yeasts; lactic acid bacteria



**Citation:** Ben Cresciuto, G.F.; Mandalà, C.; Migliori, C.A.; Cortellino, G.; Vanoli, M.; Bardi, L. Assessment of Starters of Lactic Acid Bacteria and Killer Yeasts: Selected Strains in Lab-Scale Fermentations of Table Olives (*Olea europaea* L.) cv. *Leccino*. *Fermentation* **2023**, *9*, 182. <https://doi.org/10.3390/>

IL SEGRETARIO



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