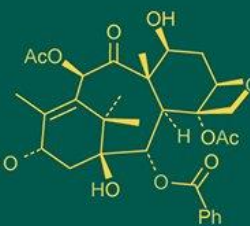
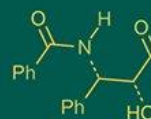
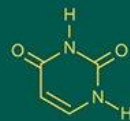


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 NAAS Rating (2026): 5.29
 IJABR 2026; 10(6): 1342-1347
www.biochemjournal.com
 Received: 02-06-2026
 Accepted: 27-06-2026

Jitesh Tarak B Shekhar
 College of Dairy Science and
 Food Technology, DSVCKV,
 Raipur, Chhattisgarh, India

B Narsimlu
 College of Dairy Science and
 Food Technology, DSVCKV,
 Raipur, Chhattisgarh, India

Praveen Kumar Tiwari
 College of Dairy Science and
 Food Technology, DSVCKV,
 Raipur, Chhattisgarh, India

Dr. Sarang
 College of Dairy Science and
 Food Technology, DSVCKV,
 Raipur, Chhattisgarh, India

Dr. Manorama
 College of Dairy Science and
 Food Technology, DSVCKV,
 Raipur, Chhattisgarh, India

Corresponding Author:
Jitesh Tarak B Shekhar
 College of Dairy Science and
 Food Technology, DSVCKV,
 Raipur, Chhattisgarh, India

Respiration-competent indigenous *Lactobacillus* from traditional Dahi and plant sources: Functional characterization for dairy industry applications

Jitesh Tarak B Shekhar, B Narsimlu, Praveen Kumar Tiwari, Sarang and Manorama

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i6p.8955>

Abstract

Lactic acid bacteria remain central to the manufacture of fermented foods, yet conventional propagation of lactobacilli under fermentative conditions can limit biomass yield and downstream robustness. Recent work on selected lactobacilli has shown that oxygen, when paired with heme and menaquinone supplementation, can activate a respiration-like metabolism that improves growth and stress tolerance in strain-dependent ways. Building on a previously generated collection of indigenous isolates from dairy and plant-associated sources in Chhattisgarh, this study is reframed here as an application-oriented evaluation of respiration competence for starter-culture development rather than as a simple isolation survey. Thirty nine phenotypically confirmed *Lactobacillus* isolates were available from dahi, vegetables, and fruit-associated samples, and their behavior under standard MRS and respiration-promoting MRS supplemented with heme and menaquinone was compared using optical density as a preliminary screen. Twenty-three isolates showed improved growth under the supplemented condition, indicating that respiration competence was widespread but strongly strain dependent, while isolate Gjr exhibited the highest response under static cultivation with an OD₆₀₀ increase of 0.662 over the corresponding control. The reframed interpretation of these findings is that indigenous lactobacilli can be triaged into respiration-responsive and nonresponsive groups for subsequent starter optimization, including high-biomass inoculum production, improved oxidative robustness, and potentially better survival during preservation steps such as refrigerated storage or freeze-drying. The study therefore supports a transition from descriptive screening toward respiratory conditioning as a practical strategy for developing robust starter and adjunct cultures from local microbial resources.

Keywords: Respiration-competent *Lactobacillus*, indigenous starter cultures, heme/menaquinone supplementation, functional dairy applications, biomass yield, stress robustness

Introduction

Species within the genus *Lactobacillus* have a long history of safe consumption and are globally recognized as premier probiotic microorganisms owing to their well-documented health benefits ^[1]. Beyond their therapeutic value, indigenous *Lactobacilli* are deeply intertwined with the natural microbial ecology of fermented foods, fresh plant matrices, and traditional dairy ecosystems. Despite their ubiquity, many of these native, wild-type strains possess highly desirable technological and functional traits that remain largely unexploited for commercial starter culture development and industrial bioprocessing. In modern dairy manufacturing, the commercial exploitation of these native strains requires comprehensive evaluation of their industrial robustness. The ultimate commercial viability of an industrial starter culture depends not only on primary acidification kinetics but also on critical processing parameters, including high biomass productivity, long-term storage stability, oxidative stress tolerance, and robust survival during rigorous downstream preservation techniques such as freeze-drying. These requirements have renewed interest in metabolic strategies that can enhance the robustness of lactic acid bacteria before their deployment in industrial systems ^[2]. *Lactobacilli* have traditionally been regarded as primarily fermentative organisms, relying heavily on substrate-level phosphorylation and generally lacking the complete respiratory machinery found in classical aerobic bacteria. However, this perspective has changed substantially. Several strains are now known to adopt a respiration-

like metabolism when oxygen is available in conjunction with exogenous heme and menaquinone sources, enabling the activation of electron transport-linked functions that enhance growth and physiological robustness. In *Lactobacillus plantarum* WCFS1, supplementation with both heme and menaquinone increased biomass production and altered end-product formation, demonstrating that respiratory phenotypes can be induced through external cofactor provision. Similar benefits have been documented in *Lactobacillus casei*, where respiratory cultivation improved survival against oxidative stress and freeze-drying stress, rendering this approach attractive for industrial starter culture production^[2, 4].

The present study isolated and phenotypically characterized *Lactobacilli* from local dairy and plant samples, then screened them for growth enhancement under heme- and menaquinone-supplemented conditions. Rather than presenting this dataset as a simple catalog of isolates, the work constitutes a preliminary bioprospecting exercise for identifying starter culture candidates with respiratory potential. This perspective is essential because the central scientific and industrial question is not merely whether an isolate exhibits improved growth in enriched MRS, but whether respiratory conditioning can be leveraged to generate superior starter cultures optimized for dairy manufacturing (Ricciardi *et al.*, 2019; Zotta *et al.*, 2017). Accordingly, the objective of the present study is to characterize this indigenous collection through the lens of starter culture functionality and industrial applicability. Primary emphasis is placed on identifying respiration-responsive native *Lactobacillus* isolates, documenting phenotypic strain heterogeneity under oxidative and processing stress conditions, and recognizing top-performing candidates. Ultimately, this work demonstrates how these robust, high-performing indigenous strains can be strategically advanced toward industrial applications, including high-density concentrated inoculum production, development of innovative fermented milk products, and design of stress-resilient probiotic adjunct cultures for modern functional dairy systems^[2, 5].

Materials and Methods

Media and Reagents

All media used in the present study were prepared with distilled water and sterilized by autoclaving at 121 °C and 15 psi for 15-20 minutes. Media were obtained from Hi Media Laboratories Pvt. Ltd., Mumbai, India. Hemin was used as the heme source for inducing aerobic respiration, while menaquinone (Supelco, Bellefonte, PA, USA) was added as the electron carrier supplement in MRS broth.

Sample Collection

A total of 60 samples comprising 40 dairy samples and 20 vegetable samples were collected from rural and urban areas of Chhattisgarh, India. All samples were collected aseptically in sterile containers and transported to the laboratory for immediate processing. Dairy samples consisted of Dahi (traditional curd), while vegetable samples included cucumber, spinach, brinjal, chilli, tomato, broccoli, cabbage, ridge gourd, bottle gourd, and lady finger.

Isolation and Preliminary Phenotypic Identification

Dahi samples were homogenized in sterile saline prior to serial dilution, while vegetable samples were first enriched in MRS broth and subsequently plated following dilution. Colonies with typical translucent to whitish appearance were selected from MRS agar plates after incubation at 37 °C for 24-48 h and transferred to broth for purification. Purity was confirmed by microscopy, and isolates displaying characteristics consistent with lactic acid bacteria were retained for further investigation. Phenotypic identification employed a polyphasic approach based on cell morphology, Gram reaction, catalase activity, and carbohydrate utilization patterns. Gram staining revealed that all selected isolates were Gram-positive rods, consistent with the morphology expected for *Lactobacillus* species. Catalase activity was tested with 3% hydrogen peroxide to differentiate typical catalase-negative lactic acid bacteria from unrelated contaminants. Carbohydrate fermentation profiles were generated using a commercial Hi-Carbo kit containing twelve immobilized carbohydrates. Based on this polyphasic characterization, 36 isolates were retained as *Lactobacillus* cultures for further physiological evaluation.

Assessment of Respiration-Competent *Lactobacillus* Isolates

Respiration-promoting medium was prepared by supplementing MRS broth with 2.5 µg/ml hemin and 1.0 µg/ml menaquinone. Hemin stock was prepared in 0.05 M NaOH, while menaquinone stock was prepared in ethanol and added aseptically following sterilization of the broth. This formulation is consistent with respiration-inducing conditions employed in related literature for selected *Lactobacilli*.

Functional Screening for Respiratory Response

Freshly activated cultures were inoculated at 2% v/v into unsupplemented MRS broth and into heme- and menaquinone-supplemented MRS (MRS-HM) for comparative growth assessment. Incubation was performed at 37 °C under both static and shaking conditions, and growth was evaluated spectrophotometrically (UV-Vis 119, Systronics Pvt. Ltd. Ahmedabad, India) at OD600 following incubation. OD600 measurement was used as a preliminary screening metric for respiratory responsiveness rather than as a definitive measure of culture performance. Isolates exhibiting higher optical density under supplemented conditions were categorized as respiration-responsive and selected as candidates for future starter culture development based on their demonstrated respiratory competence.

Data Interpretation Strategy

The original dataset emphasized isolate-level differences in OD600 between control and supplemented conditions. In the present analysis, these data are interpreted functionally. A positive growth differential in the presence of hemin and menaquinone is interpreted as evidence of respiratory conditioning potential, whereas negligible or negative responses indicate strain-specific non-responsiveness or hemin sensitivity, as also documented in the literature. The strongest-performing isolates are therefore positioned as candidates for subsequent application studies rather than as standalone endpoints.

Results and Discussion

Isolation of indigenous *Lactobacilli* from dairy and plant-associated niches

The isolation procedure yielded presumptive lactic acid bacteria from Dahi and plant-associated samples collected from different locations of Chhattisgarh. Following purification and phenotypic characterization, 36 isolates were identified as members of the genus *Lactobacillus* based on their Gram-positive rod morphology, predominantly catalase-negative reaction, and carbohydrate fermentation profiles characteristic of the genus as presented in Table 1 and Figure 1. The successful recovery of these isolates confirms that traditional fermented dairy products and fresh plant materials constitute valuable natural reservoirs of indigenous *Lactobacillus* strains with potential technological significance. This observation is consistent with previous reports demonstrating that naturally fermented dairy products harbor diverse populations of *Lactobacillus* because spontaneous fermentation, nutrient-rich milk composition, and acidic conditions selectively favor the growth of lactic acid bacteria [6, 7]. Similarly, fresh vegetables and other plant materials have been recognized as important ecological niches for epiphytic lactobacilli, where these bacteria adapt to fluctuating environmental conditions such as oxygen availability, nutrient limitation, and variable pH [8, 9].

Respiration screening on isolates

All the 36 phenotypically identified isolates were successfully revived and subjected to respiration-promoting growth conditions using MRS broth supplemented with heme and menaquinone. Fourteen isolates exhibited a marked increase in optical density compared with the unsupplemented control, indicating that approximately 39% of the indigenous *Lactobacillus* collection benefited from respiratory conditioning. The heterogeneous growth responses observed in the present study further emphasize the strain-specific nature of respiratory metabolism. While several isolates exhibited substantial increases in optical density under supplemented conditions, others showed only marginal improvement or even reduced growth relative to the control. Such variability has also been reported by (2), who demonstrated that only selected *Lactobacillus* strains exhibited significant respiratory enhancement following heme and quinone supplementation, whereas other strains remained unaffected despite belonging to the same genus.

Superior Respiratory Growth Potent Isolate

Among the respiration-responsive isolates evaluated under heme- and menaquinone-supplemented MRS broth, isolate Krb2 exhibited the highest growth response, attaining an OD₆₀₀ of 1.054 compared with 0.501 in the corresponding unsupplemented control. This marked increase in biomass indicates that Krb2 possesses a highly efficient respiratory metabolism under the tested conditions, enabling improved energy conservation and enhanced cell proliferation during aerobic cultivation. Respiratory metabolism in certain *Lactobacillus* strains is known to enhance ATP generation through the establishment of a functional electron transport chain in the presence of heme and quinone, thereby promoting higher biomass yields and increased tolerance to oxidative stress [2, 4]. The superior performance of Krb2 therefore suggests that this isolate may possess the genetic and physiological machinery required to efficiently utilize

respiratory cofactors, making it a promising candidate for industrial biomass production. Increased biomass generation is particularly advantageous for starter culture manufacture, as it can improve inoculum yield, reduce production costs, and enhance the viability of cultures during downstream processing [10].

Besides Krb2, isolates jdpb, dmtr, Surj, and Chil nw also exhibited appreciable increases in optical density under respiration-promoting conditions, indicating that respiratory competence is distributed among several indigenous isolates rather than being restricted to a single strain. Such variability is expected because respiratory capacity in *Lactobacillus* is a strain-dependent characteristic influenced by differences in respiratory gene clusters, cofactor utilization, and oxidative stress response systems [2, 3]. The identification of multiple respiration-responsive isolates is advantageous from a technological perspective because selection of starter cultures should consider several functional traits in addition to biomass production. Although Krb2 demonstrated the highest growth response, other isolates may possess superior characteristics with respect to acidification rate, flavor compound production, stress tolerance, or probiotic functionality. Similar observations have been reported by Ricciardi *et al.* [5], who emphasized that the technological suitability of lactic acid bacteria depends on the combined expression of multiple desirable traits rather than a single growth parameter. Therefore, the present findings support the development of a diverse panel of indigenous starter candidates for subsequent technological characterization instead of relying exclusively on a single isolate.

Respiratory Competence Distribution across Multiple Indigenous Isolates

Comparison of cultivation conditions further revealed that respiratory enhancement was generally more pronounced during static incubation than under shaking conditions. Several isolates exhibited greater differences between supplemented and control cultures in static cultures, whereas agitation either reduced the magnitude of the response or eliminated it altogether. This observation suggests that oxygen availability and aeration must be carefully balanced to achieve optimal respiratory metabolism. Moderate oxygen levels supplied under static cultivation may be sufficient to activate the electron transport chain in respiration-competent lactobacilli, whereas excessive oxygen transfer during shaking can increase the generation of reactive oxygen species, thereby imposing oxidative stress that counteracts the beneficial effects of respiratory metabolism [2, 4]. Similar findings have been reported by Zotta *et al.* [3], who demonstrated that respiratory performance in lactic acid bacteria is strongly influenced by the interaction between oxygen concentration, heme availability, and redox balance. Consequently, the expression of respiratory metabolism is not determined solely by supplementation with heme and menaquinone but also depends on cultivation parameters such as aeration, agitation rate, inoculum density, and medium composition. These findings highlight the importance of process optimization for maximizing biomass production in respiration-competent indigenous *Lactobacillus* strains and provide a basis for future bioprocess studies aimed at improving starter culture propagation under controlled aerobic conditions.

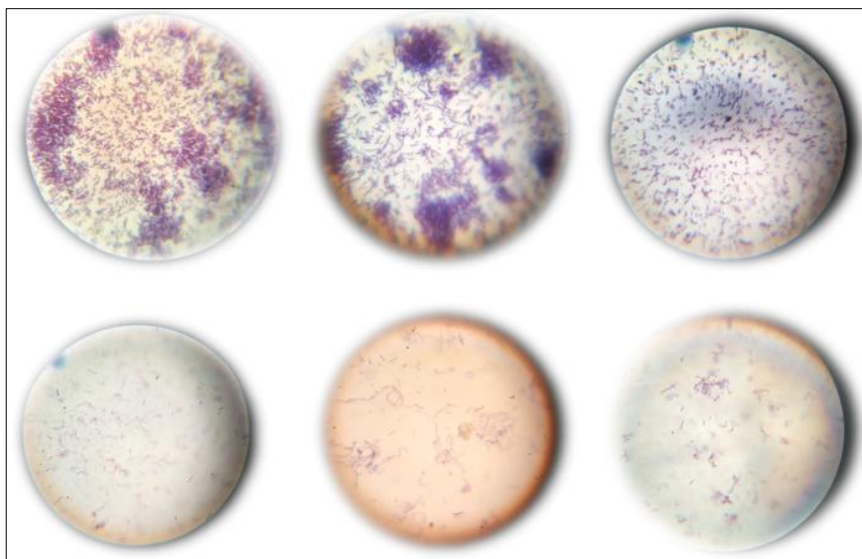


Fig 1: Gram staining and colony morphology of selected isolates

Table 1: Carbohydrate utilization pattern (sugar profiling) of isolates

Isolates	Lact	Xyl	Malt	Fru	Dext	Gala	Raffi	Treh	Meli	Sucr	L-Arb	Man
Jora	+	+	+	+	+	+	+	+	+	+	+	+
Nilg	+	+	+	+	+	+	+	+	+	+	+	+
Jdp	+	+	+	+	+	+	+	+	+	+	+	+
Jdpb	+	+	+	+	+	+	+	+	+	+	+	+
Mndr	+	+	-	+	+	+	+	-	+	+	+	+
Ishw	+	+	+	+	+	+	+	+	+	+	+	+
Dmtr	+	+	+	+	+	+	+	+	+	+	+	+
Dlrj	+	+	-	+	+	+	+	-	+	+	+	+
Surj	+	-	+	+	+	+	+	+	+	+	-	+
Rjn	+	+	+	+	+	+	+	+	+	+	+	+
Abk	+	+	+	+	+	+	+	+	+	+	+	+
Saho	+	+	+	+	+	+	+	+	+	+	+	+
Rai	+	+	+	+	+	+	+	+	+	+	+	+
Durg	+	+	+	+	+	+	+	+	+	+	+	+
Kchn	+	+	+	+	+	+	+	+	+	+	+	+
Dist-R	+	+	+	+	+	+	+	+	+	+	+	+
Gingr	+	+	+	+	+	+	+	+	+	+	+	+
Korb	+	+	+	+	+	+	+	+	+	+	+	+
Hari	+	+	+	+	+	+	+	+	+	+	+	+
Bsp	+	+	+	+	+	+	+	+	+	+	+	+
Gari-2	+	+	+	+	+	+	+	+	+	+	+	+
Bilha	+	+	+	+	+	+	+	+	+	+	+	+
Gari-1	+	+	+	+	+	+	+	+	+	+	+	+
Korb2	+	-	+	+	+	+	+	+	+	+	+	+
Gri2	+	+	+	+	+	+	+	+	+	+	+	+
Rpr-s	+	+	+	+	+	+	+	+	+	+	+	+
Tilda	+	+	+	+	+	+	+	+	+	+	+	+
Lf jora	+	+	+	+	+	+	+	+	+	+	+	+
Chil nw	+	+	+	+	+	+	+	+	+	+	+	+
Saja	+	+	+	+	+	+	+	+	+	+	+	+
Gudyr	+	+	+	+	+	+	+	+	+	+	+	+
GudyrB	+	+	+	+	+	+	+	+	+	+	+	+
Dhmda	+	+	+	+	+	+	+	+	+	+	+	+
Lalpr	+	+	+	+	+	+	+	+	+	+	+	+
Ccbr Ab	+	+	+	+	+	+	+	+	+	+	+	+
L.fmtm	+	+	-	+	+	+	+	+	+	-	+	+
Cabge nr	+	+	+	+	+	+	+	+	+	+	+	+
Lf nr	+	+	+	+	+	+	+	+	+	+	+	+
Ccbr nr	+	+	+	+	+	+	+	+	+	+	+	+

Table 2: Strain-dependent response to respiration-promoting conditions

S.no	Culture name	Shaking		Static	
		Control	Treatment (H+M)	Control	Treatment (H+M)
1	Jora	0.624±0.005	0.634±0.003	0.633±0.002	0.635±0.006
2	Jdp	0.576±0.003	0.551±0.004	0.558±0.004	0.556±0.003
3	Jdpb	0.505±0.002	0.771±0.001	0.537±0.001	0.842±0.001
4	Mndr	0.667±0.006	0.867±0.004	0.613±0.015	0.678±0.005
5	Dmtr	0.614±0.003	0.666±0.001	0.626±0.005	0.813±0.003
6	Dlrj	0.517±0.008	0.627±0.004	0.631±0.011	0.725±0.002
7	Surj	0.664±0.007	0.683±0.009	0.685±0.011	0.817±0.006
8	Rjn	0.420±0.002	0.417±0.001	0.526±0.005	0.554±0.004
9	Abk	0.475±0.008	0.443±0.017	0.507±0.005	0.506±0.006
10	Sahd	0.485±0.015	0.436±0.035	0.545±0.001	0.503±0.002
11	Durg	0.599±0.005	0.431±0.004	0.595±0.003	0.622±0.010
12	Kchn	0.593±0.006	0.709±0.002	0.605±0.330	0.869±0.007
13	Dist-R	0.485±0.001	0.496±0.002	0.418±0.001	0.486±0.002
14	Gingr	0.528±0.005	0.456±0.003	0.452±0.008	0.593±0.007
15	Korb	0.565±0.008	0.589±0.010	0.571±0.004	0.593±0.001
16	Hari	0.475±0.0003	0.451±0.003	0.419±0.003	0.435±0.003
17	Bsp	0.566±0.002	0.603±0.007	0.577±0.010	0.543±0.006
18	Gari-2	0.358±0.001	0.340±0.003	0.331±0.008	0.309±0.002
19	Bilha	0.677±0.006	0.617±0.004	0.894±0.005	0.923±0.012
20	Gari-1	0.430±0.001	0.422±0.002	0.891±0.002	0.876±0.003
21	Krb2	0.501±0.001	1.054±0.001	0.513±0.005	0.812±0.005
22	Gri2	0.558±0.002	0.547±0.026	0.545±0.002	0.540±0.006
23	Rpr-s	0.617±0.002	0.623±0.007	0.652±0.005	0.638±0.003
24	Tilda	0.628±0.001	0.622±0.004	0.624±0.002	0.626±0.033
25	Lf jora	0.545±0.004	0.511±0.009	0.516±0.001	0.505±0.003
26	Chil nw	0.515±0.003	0.724±0.010	0.514±0.002	0.912±0.006
27	Saja	0.787±0.006	0.603±0.004	0.690±0.004	0.616±0.001
28	Gudyr	0.430±0.005	0.651±0.018	0.548±0.004	0.632±0.0003
29	GudyrB	0.570±0.003	0.560±0.002	0.530±0.003	0.503±0.004
30	Dhmda	0.524±0.001	0.730±0.0003	0.532±0.014	0.603±0.012
31	Lalpr	0.592±0.002	0.562±0.011	0.502±0.002	0.501±0.002
32	Ccbr Ab	0.479±0.332	0.912±0.004	0.421±0.008	0.537±0.003
33	L.fmtm	0.561±0.072	0.949±0.004	0.512±0.004	0.611±0.002
34	Cabge nr	0.453±0.007	0.426±0.009	0.504±0.003	0.502±0.011
35	Lf nr	0.507±0.004	0.655±0.001	0.564±0.003	0.754±0.0006
36	Ccbr nr	0.567±0.006	0.693±0.002	0.572±0.009	0.601±0.007

Results are expressed as Mean ± SE (n=3)

Conclusion

This study interprets as a functional bioprospecting effort for starter-culture improvement rather than only a descriptive isolation exercise. Among 36 phenotypically confirmed *Lactobacillus* isolates recovered from Dahi and plant-associated samples, 14 displayed improved growth under heme- and menaquinone-supplemented conditions, demonstrating that respiratory responsiveness is common but clearly strain dependent in indigenous *Lactobacilli*. Isolate Krb2 showed the strongest response and therefore represents a priority candidate for deeper evaluation as a high-biomass and potentially stress robust culture. Taken together with published evidence that respiratory cultivation can improve biomass yield, oxidative tolerance, and preservation survival in selected *Lactobacilli*, these findings support respiratory conditioning as a practical platform for developing stronger starter and adjunct cultures from local microbial resources. The study thus lays a foundation for a next generation of work focused on molecular confirmation, process optimization, and dairy performance validation under starter-manufacturing conditions.

References

- Pandey KR, Naik SR, Vakil BV. Probiotics, prebiotics and synbiotics: a review. *J Food Sci Technol*. 2015;52(12):7577-7587. DOI:10.1007/s13197-015-1921-1.
- Ianniello RG, Zotta T, Matera A, Genovese F, Parente E, Ricciardi A. Investigation of factors affecting aerobic and respiratory growth in the oxygen-tolerant strain *Lactobacillus casei* N87. *PLoS One*. 2016;11(11):e0164065. DOI:10.1371/journal.pone.0164065.
- Zotta T, Parente E, Ricciardi A. Aerobic metabolism in the genus *Lactobacillus*: impact on stress response and potential applications in the food industry. *J Appl Microbiol*. 2017;122(4):857-869. DOI:10.1111/jam.13399.
- Brooijmans RJW, de Vos WM, Hugenholtz J. *Lactobacillus plantarum* WCFS1 electron transport chains. *Appl Environ Microbiol*. 2009;75(11):3580-3585. DOI:10.1128/AEM.00147-09.
- Ricciardi A, Zotta T, Ianniello RG, Boscaino F, Matera A, Parente E. Effect of respiratory growth on the metabolite production and stress robustness of *Lactobacillus casei* N87 cultivated in cheese whey permeate medium. *Front Microbiol*. 2019;10:851. DOI:10.3389/fmicb.2019.00851.
- Tamang JP, Watanabe K, Holzapfel WH. Diversity of microorganisms in global fermented foods and

- beverages. *Front Microbiol.* 2016;7:377. DOI:10.3389/fmicb.2016.00377.
7. Leroy F, De Vuyst L. Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends Food Sci Technol.* 2004;15(2):67-78. DOI:10.1016/j.tifs.2003.09.004.
 8. Di Cagno R, Coda R, De Angelis M, Gobbetti M. Exploitation of vegetables and fruits through lactic acid fermentation. *Food Microbiol.* 2013;33(1):1-10. DOI:10.1016/j.fm.2012.09.003.
 9. Filannino P, Di Cagno R, Gobbetti M. Metabolic and functional paths of lactic acid bacteria in plant foods: get out of the labyrinth. *Curr Opin Biotechnol.* 2018;49:64-72. DOI:10.1016/j.copbio.2017.07.016.
 10. Bintsis T. Lactic acid bacteria as starter cultures: an update in their metabolism and genetics. *AIMS Microbiol.* 2018;4(4):665-684. DOI:10.3934/microbiol.2018.4.665.