INNSPUB

**International Journal of Biosciences | IJB |**
**ISSN: 2220-6655 (Print); 2222-5234 (Online)**
**Website: https://www.innspub.net**
**Email contact: info@innspub.net**
**Vol. 27, Issue: 1, p. 297-305, 2025**

RESEARCH PAPER OPEN ACCESS

# Postbiotic cell free supernatant from *Lactobacillus* strain: Isolation, characterization, *In vitro* assessment of antimicrobial and cytotoxic potentials using Vero cell line

**Arunavarsini Kumarasamy[1], R. Sornambiga[2], Mahenthiran Ramasamy[*1]**

[1]*Department of Microbiology, Dr. N.G.P. Arts and Science College, Coimbatore, India*

[2]*Department of Microbiology, PSG College of Arts and Science, Coimbatore, India*

**Key words:** Yogurt, Postbiotics, Cell-free supernatant, Probiotic bacteria, Antimicrobial resistance

DOI: https://dx.doi.org/10.12692/ijb/27.1.297-305 Published: July 15, 2025

**ABSTRACT**

This study investigates the isolation, identification, and characterization of a Lactobacillus paracasei strain derived from homemade yogurt, emphasizing its potential as a source of bioactive postbiotics. Using morphological and biochemical methods, including MALDI-TOF MS, the strain was reliably identified at the genus and species levels. The probiotic nature of the isolate was confirmed through culture and microscopic analysis. The research further explored the production of postbiotic cell-free supernatants from the isolated strain, assessing their antimicrobial and cytotoxic activities. The postbiotics demonstrated significant inhibitory effects against pathogenic bacteria such as *Escherichia coli*, *Salmonella enterica*, *Streptococcus bovis*, and *Enterococcus faecalis*, often exhibiting antimicrobial efficacy comparable or superior to standard antibiotics. The safety profile was affirmed by cytotoxicity assays on Vero cell lines, underscoring their low toxicity and potential for safe therapeutic application. The findings support the notion that postbiotics hold promising prospects as natural antimicrobial agents, especially in light of rising antibiotic resistance. Their stability and ease of handling offer advantages over live probiotics. The study emphasizes the importance of further clinical investigations and mechanistic studies to optimize the application of postbiotics in medical and food industry contexts, promoting safer and more effective health interventions.

***Corresponding author:** R. Mahenthiran ✉ arunavarsini@gmail.com

## INTRODUCTION

Yogurt is a popular milk product that is made by fermenting milk with the help of specific probiotic bacterial cultures. The process not only thickens the milk but also imparts a tangy flavor and enhances the nutritional value by forming beneficial compounds, such as vitamins and bioactive peptides. The probiotic character of yogurt is attributed to the live microorganisms that can impart a range of health benefits when consumed in adequate amounts. Recent research also points towards the inclusion of prebiotics and synbiotics in yogurt, thereby enhancing its functional properties (Olson and Kayanush, 2022). The inclusion of such ingredients enables the development of new yogurt products that are designed to provide maximum health benefits while meeting different consumer needs.

The consumption of probiotics, which are live microbes that offer health benefits to the host when taken in sufficient amounts, is essential. First coined from the Greek word meaning "for life," probiotics are mostly made up of certain bacteria strains, i.e., *Lactobacillus* and *Bifidobacterium*, which are crucial for gut health and overall well-being. Traditionally known for supporting gut health, probiotics help to promote a healthy gut microbiota, necessary for digestion, nutrient uptake, and immune response. New studies have extended the uses and benefits of probiotics and their link to the improvement of many conditions, such as irritable bowel syndrome, lactose intolerance, and even mental illness via the gut-brain axis. Aside from traditional probiotics, other relevant new ideas, such as postbiotics, have made headlines for their possible benefits (Zendeboodi *et al.*, 2020).

Postbiotics are bioactive molecules derived from a live microorganism via fermentation or metabolic activity that have positive health impacts on the host. "Postbiotics" is a term used to describe a variety of materials, which are dissolved cell components, metabolites, and other non-viable compounds such as peptides, polysaccharides, bacteriocins, and organic acids. Unlike probiotics, which consist of live bacteria that must be carefully cultivated and stored, postbiotics can come from non-viable microorganisms or their components. This renders it more stable and easier to handle, as postbiotics do not require strict processing and storage requirements (Bourebaba *et al.*, 2022).

Postbiotics has positive benefits through multiple mechanisms of action towards host well-being. First, postbiotics play a role in gut microbiota modulation by stimulating the growth of health-promoting microbial populations at the expense of pathogenic microorganisms and thus attaining a balanced microbiome. Second, postbiotics help enhance intestinal barrier function, which plays a significant role in preventing the translocation of toxic agents into the bloodstream. They achieve this by promoting the expression of tight junction proteins and secretory compounds that protect against the epithelial lining. Postbiotics are also immunomodulatory in nature; they activate immune cells and control cytokine production, resulting in a perfectly coordinated immune response that can prevent inflammation (Hijová, 2024).

Cell-free supernatants (CFS) are liquid extracts isolated from microbial cultures once the microbe has fermented a growth substrate, consisting of a number of metabolites, enzymes, and unused nutrients. Cell-free supernatants are known for their bioactive functions, such as antibacterial, anti-inflammatory, antioxidant, and antitumor activities (Lee *et al.*, 2022).

Cell-free supernatants provide an alternative to conventional antimicrobials that is both safe and efficient and could possibly redress concerns over antibiotic resistance. The antibacterial action is mostly due to organic acids, proteinaceous molecules, and fatty acids that are formed during fermentation (Mani *et al.*, 2022).

The study has also established (Thorakkattu *et al.*, 2022) that postbiotics have been good antimicrobials against several pathogenic

microorganisms. Postbiotics have also been found to be effective in modulating the host's immune response, as well as being very potent as an antimicrobial agent. Postbiotics have many advantages over traditional antibiotics, including a lower chance of antibiotic resistance and increased safety for vulnerable populations.

Cytotoxic activity is an important postbiotic characteristic that highlights their importance in helping to maintain cellular homeostasis and ensuring overall health. This activity points to the capacity of the postbiotics to selectively induce cell death in pathogenic or dysfunctional cells, thus facilitating the clearance of damaged or infected cells while sparing healthy tissue.

By regulating immune responses and reducing inflammation. In addition, postbiotics' cytotoxic effects facilitate tissue repair and regeneration. Thus, the knowledge of postbiotics' cytotoxic effects not only highlights their importance as prospective therapeutic agents but also justifies the verification of postbiotics (Aghebati *et al.,* 2021).

Cell line research is crucial for understanding the action of postbiotics, since cell lines offer an in vitro homogeneous and controlled environment to evaluate cell reactions to metabolites. With the use of several cell lines, scientists are able to determine mechanisms of action, bioactive nature, and therapeutic potential of postbiotics against various types of cells. Cell line research allows scientists to categorize dose-dependent effects and determine the way postbiotics affect cell processes, such as proliferation, differentiation, and metabolism (Elham *et al.,* 2022).

The current study aims to evaluate the antimicrobial activity of postbiotic cell-free supernatant, which is obtained from a Lactobacillus strain (obtained from homemade yogurt), and to perform and check the cytotoxic activity of postbiotics on Vero cell lines.

## MATERIALS AND METHODS

### Yogurt preparation

Yogurt was prepared following (Dalhan and Sani, 2017) with slight modifications. Fresh cow's milk was collected from a farm near Erode district. Milk was added and allowed to cool down. Later, a starter culture was added and kept for fermentation overnight at 37°C or in normal room temperature. After overnight fermentation of the yogurt, excess water in the yogurt was removed and it was stored at 4°C until further use.

### Isolation

Take two grams of the sample and transfer it to a flask with 100 ml of MRS broth as the enrichment medium, and incubate for 24 hours at 37°C. After 24 hours, 100 µl of the samples were spread and plated on MRS agar and incubated for 48 hours at 37°C. Subsequent subcultures were made for the bacterial colonies ( Karami *et al.,* 2017).

### Gram staining

Gram stain was done by modifying (Ariful *et al.,* 2023). Bacterial cultures were streaked onto MRS agar and incubated at 37°C for 24-48 hours. Aseptically, one isolated colony was picked and smeared onto a sterile, dry glass slide; it was then heat-fixed. The heat-fixed smear was washed with distilled water for five seconds, followed by thirty seconds in the crystal violet solution. A few drops of Gram's iodine solution were washed with five seconds of running tap water, then decolorized with 95% ethanol for 15-30 seconds, and the slide was washed finally for five seconds. Following a counterstain with safranin for 60-80 seconds, the slide was washed with water. The bacteria were observed under 100x magnification through a microscope.

### Biochemical characterization

Biochemical identification of the isolated strain was carried out by performing the tests as described by (Phani *et al.,* 2024), with some modifications. The indole test, methyl red test, Voges-Proskauer test, citrate utilization test, catalase test, and oxidase test

were conducted in accordance with Bergey's Manual of Systematic Bacteriology for all the above-mentioned biochemical tests.

**Maldi-tof**

The isolated colony has undergone MALDI-TOF MS (matrix-assisted laser desorption ionization time of flight mass spectrometry)-based VITEK MS PRIME for genus-level identification. The isolate has shown a high score value of 99.9% in the control indication (Ozbey *et al.,* 2022).

**Postbiotic cell-free supernatant preparation**

Postbiotic cell-free supernatant was prepared according to (Yang *et al.,* 2021) with slight modifications. Isolated Lactobacillus culture broth was grown in MRS broth for 15 minutes at 4000 rpm; the supernatant was collected. Then, the collected supernatant was filtered through a 0.22 µm membrane filter to remove the excess bacterial cell debris. The postbiotic cell-free supernatant is now stored at 4°C until use.

**Antimicrobial sensitivity**

The antagonistic activity of postbiotic cell-free supernatant was adopted from (Kaewchomphunuch *et al.,* 2022) with few modifications. The antimicrobial sensitivity test, agar well diffusion, was performed to evaluate the inhibitory activities against pathogenic *Escherichia coli*, *Streptococcus bovis*, *Salmonella enterica,* and *Enterococcus faecalis*.

Initially, Muller Hinton agar was prepared and poured into sterile plates, and 24-hour incubated bacterial strains were swabbed into the MHA plates. Postbiotic samples were loaded into each well at 25 µl, 50 µl, 75 µl, and 100 µl, along with standard antibiotic discs, and incubated at 37°C. To evaluate the result, the zone of inhibition in diameter (mm) was measured.

**Cell lines**

The Vero cell (normal kidney epithelial cells of the African green monkey) is acquired from the National Centre for Cell Sciences (NCCS), Pune, India.

**Maintenance of Vero cell lines**

The normal kidney epithelial cells of the African green monkey (Vero) are the cell type used in this study on a DMEM M (HiMedia) medium supplemented with 10% fetal bovine serum (HiMedia) along with 1% antibiotic-antimycotic solution. In a $CO_2$ incubator, the cells were cultivated until confluent at 37°C (Ammerman *et al.,* 2008).

**Preparation of Vero cell lines**

Followed by the incubation process of cell culture in the $CO_2$ incubator according to (Widiastutu *et al.*, 2023), a fresh medium was added, and trypsin was subsequently dripped over the cells to harvest them. After that, the collected Vero cells were re-cultured at an initial density of 2 x 10^2 cells/100 mL in a 96-well plate. After 24 hours of incubation, the Vero cell lines were nearly confluent. In order to avoid the Vero cell line culture from becoming highly packed, postbiotic cell-free supernatant was added to the microplate after adding the culture.

**Cytotoxicity assay**

The cytotoxic potential of the postbiotic cell-free supernatant was evaluated using the MTT assay on Vero cells, as described by (Piaru *et al.,* 2012). Initially, Vero cells were harvested from culture flasks and seeded into 96-well plates at a density of 20,000 cells per well, with 100 µL of fresh growth medium added to each well, without the test agent. The cells were allowed to adhere and incubate for 24 hours under standard conditions. Subsequently, the cells were treated with serially diluted concentrations of postbiotic cell-free supernatant, prepared by diluting the stock solution in growth medium. After 24 hours of incubation, the medium was carefully removed, and the cells were washed once with sterile phosphate-buffered saline (PBS). Then, 0.5 mg/mL of MTT reagent dissolved in PBS was added to each well, and the plates were incubated at 37°C in a 5% $CO_2$ atmosphere for 2 to 4 hours. The medium was then discarded, and 100 µL of DMSO was added to each well to solubilize the formazan. The plates were gently agitated for 5 minutes to ensure complete dissolution,

especially in dense cultures. The absorbance of each well was measured at a 570 nm wavelength on a spectrophotometer, and the percentage of cell viability was calculated relative to the untreated control by using the formula below:

% cell viability = [Mean abs of treated cells/Mean abs of Untreated cells] × 100.

## RESULTS

### Morphological and microscopic observation

The morphological characteristics of the isolated strain were observed the growth on MRS agar, and the colonies appeared 24-48 hours after incubation at 37°C under anaerobic conditions. The colonies were creamy white in colour with a smooth edge. In microscopic observation under oil immersion (100x) revealed the presence of rod shaped bacilli, which were gram-positive, present in pairs. The ability to retain the crystal violet stain with the characteristic purple cells which indicated they were gram- positive due to the thick peptidoglycan layer in their cell walls (Table 1).

**Table 1.** Microscopic and morphological characterization

| Tests | Observation |
|---|---|
| Gram staining | + ve |
| Microscopic observation | Rod shaped |
| Arrangement | Short chains |
| Colony appearance | Creamy white |

### Biochemical identification

The biochemical characterization of the isolated strains was performed as follows (Table 2) the indole test was negative, which suggests that the isolated strain does not possess the capability to degrade tryptophan into indole. In the MR test, there was a positive result, indicating that the organism uses a mixed acid fermentation pathway that leads to the stable production of acidic end-products of glucose metabolism. The VP test was negative, which means that acetoin is not formed during glucose fermentation in this strain. The test of citrate utilization showed a negative result, indicating that the isolated strain cannot grow on citrate as a sole carbon source. Both catalase and oxidase tests were negative. The lack of catalase activity is typical for lactic acid bacteria, which do not produce this enzyme as a rule.

**Table 2.** Biochemical characterization of the isolate

| Biochemical tests | Results |
|---|---|
| Indole | -ve |
| Methyl red | +ve |
| Voges Proskauer | -ve |
| Citrate utilization | -ve |
| Catalase | -ve |
| Oxidase | -ve |

Likewise, the oxidase test also indicated a negative result, meaning that they lack cytochrome c oxidase in the organism.

### Maldi-tof

The bacterial isolate was analyzed at the genus level by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) through the VITEK MS PRIME system. The resulting analysis showed a high identification score of 99.9%, indicating a high match to database reference spectra. The isolate was identified from the mass spectral profile as *Lactobacillus paracasei*, and the taxonomic assignment was verified at high reliability.

### Antimicrobial activity

The antimicrobial capacity of postbiotic samples was tested against four bacterial isolates:

*Escherichia coli, Streptococcus bovis, Salmonella enterica, and Enterococcus faecalis,* using the well diffusion method. The areas of prohibition were analyzed in four postbiotic extract concentrations: 25 µl, 50 µl, 75 µl, and 100 µl. The results were made comparable with the results of standard antibiotics, which were in the form of positive control for each microorganism (Table 3).

In the case of *Escherichia coli*, postbiotic extracts showed an increase in antibacterial activity at a dose. The minimum area of prohibition was 9 mm at 25 µl and increased to 12 mm at 50 µl, 75 µl got 15

mm, and 17 mm at 100 µl. This reference was slightly higher than that produced by the antibiotic penicillin, which was 16 mm.

For *Streptococcus bovis*, no zone of inhibition was seen in the lowest volume of postbiotic samples at 25 µl. The activity of the sample increased with concentration at 10 mm for 50 µl, 12 mm for 75 µl, and 15 mm for 100 µl. The results were absolutely controlled according to the activity of the antibiotic vancomycin, which was 15.5 mm.

Although no zone of inhibition was observed at 25 µl in *Salmonella enterica*, inhibition zone was seen at 50 µl and more. For 50 µl, 75 µl, and 100 µl, the preventive areas were 8 mm, 11 mm, and 16 mm, respectively. The results were near azithromycin control, which caused the 17-mm area prohibition.

**Table 3.** Antimicrobial activity of the postbiotics cell free supernatant

| Micro-organisms | Zone of inhibition of postbiotic sample and standard antibiotics | | | | | |
|---|---|---|---|---|---|---|
| | 25 µL | 50 µL | 75 µL | 100 µL | Standard antibiotics | ZOI of antibiotics (mm) |
| *Escherichia coli* | 9 | 12 | 15 | 17 | Penicillin | 16 |
| *Streptococcus bovis* | - | 10 | 12 | 15 | Vancomycin | 15.5 |
| *Salmonella enterica* | - | 8 | 11 | 16 | Azithromycin | 17 |
| *Enterococcus faecalis* | - | 11 | 13 | 15.8 | Ampicillin | 18 |

**Table 4.** Cell viability of postbiotic sample on Vero cell lines

| Concentration of the postbiotic sample | % of cell viability |
|---|---|
| 12.5 µg/ml | 98.67 |
| 25 µg/ml | 97.23 |
| 50 µg/ml | 96.20 |
| 100 µg/ml | 94.88 |
| 150 µg/ml | 94.03 |

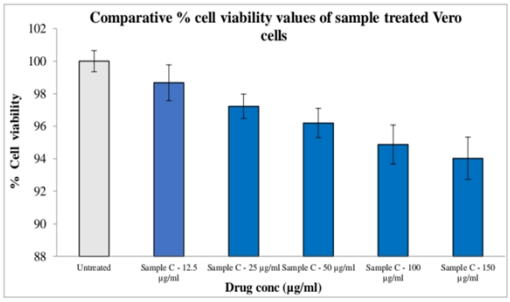


**Fig. 1.** Percentage of cell viability along with treated Vero cell lines

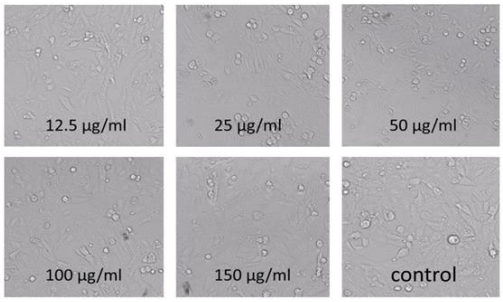


**Fig. 2.** Cytotoxic evaluation of postbiotic CFS in Vero cell lines

For the *Enterococcus faecalis*, the postbiotic extract was not active at 25 µl but was highly active in high volume. Inhibition zone increased from 50 µl as 11mm then 13 mm at 75 µl and 15.8 mm at 100 µl. However low, this activity was compared to the control drug ampicillin, with an 18 mm area.

Generally, findings suggest that postbiotic samples have high antibacterial activity, with clear doses dependent on the clear dose. In high concentrations, especially 100 µl, postbiotics performed in prohibited areas, which was comparatively better than traditional antibiotics. This suggests that postbiotic preparation can be an effective alternative or supplementary antimicrobial agent for the treatment of infections caused by particularly used bacterial strains.

**Cytotoxic assay**

Detailed cytotoxicity evaluation in the study has shown that postbiotic samples have a low capacity for toxicity for Vero cells, which are a standard cell line used to assess cytotoxic effects. The test was conducted using various concentrations of postbiotic extracts—including 12.5 µg/ ml, 25 µg/ ml, 50 µg/ml, 100 µg/ml, and 150 µg/ml—to determine how different doses affect cell viability (Table 4, Fig. 1).

At the lowest concentration (12.5 µg/ml) tested, the cell viability was approximately 98.67%, indicating that almost all cells were healthy and active, with minimal effects from the postbiotic sample. As the concentration increased, the cell viability decreased slightly, but especially high—about 94.03% at 150 µg/ml, the highest dose tested (Fig. 1). This high level of cell viability in all tested concentrations suggests that extracts do not cause significant cell damage or toxicity.

In addition, the data indicates that postbiotics have a dose-dependent effect, which means that as the dose increases, there is a slight decline in cell viability within the limit accepted for safety. The fact that cell viability remains above 94% even at the highest concentration indicates that at the level of the compound, Vero cells are relatively safe and are unlikely to induce cytotoxic effects in cells (Fig. 2). Thus, the study concludes that postbiotics have low cytotoxic capacity, which also supports their possible use in applications.

**DISCUSSION**

The inclusion of curd fermentation and subsequent probiotic strains is usually displayed by the practical provision of the production of bioactive postbiotics from foods consumed, which increases their acceptance and integration in functional foods. Positive modulation of intestine microbiota, increased intestinal obstruction functions, and immunomodulatory effects associated with postbiotics keep them in position as valuable components of microbiome-targeted interventions.

Microscopic and molecular identification confirmed the Lactobacillus species; usage of MALDI-TOF MS provided reliable genus-level identification with a guaranteed replica of the source organism and specificity as Lactobacillus paracasei. Preparation of cell-free supernatant guaranteed bioactivity, underlining the potential for direct utilization of postbiotics from fermented food like yogurt.

The results support earlier findings that can act as powerful antimicrobial substances with bioactive molecules, such as organic acids, peptides, and bacteriocins, such as postbiotics, dosage activity.

This research has shown that postbiotic drugs of Lactobacillus strains are likely to have pathogenic antimicrobial activities against such bacteria as *Escherichia coli*, *Streptococcus bovis*, *Salmonella enterica*, and *Enterococcus faecalis*. The postbiotic extracts exhibited antimicrobial potency that was similar, or even superior, to that of the commonly used antibiotics penicillin, vancomycin, and azithromycin. These findings suggest the adjunct or alternative therapeutic applications of postbiotics, particularly in response to the increase in antibiotic resistance. The enhancement of inhibition zones with increasing concentrations particularly indicates the need for the optimization of dose for optimal antimicrobial activity.

At more than 94% viability even at the highest concentration tested, the cytotoxicity test showed that postbiotics are not very harmful to Vero cells. Their safety for potential therapeutic application is corroborated by their low profile of cytotoxicity. We can employ postbiotics at effective antimicrobial concentrations without compromising the integrity of host cells, as the dose-dependent reduction in cell viability was insignificant. This safety profile is in agreement with earlier studies that have demonstrated that postbiotics are well tolerated and safe overall because of their biochemical stability and lack of viability.

This study demonstrates the safety, antimicrobial efficacy, and feasibility of using postbiotics as organic biopreservatives and therapeutic products. Future research should take into account clinical verifications, meticulous mechanistic procedures, and synergistic effects on currently available antibiotics in order to optimize their use in medical industry applications.

## CONCLUSION

In the present study, a *Lactobacillus paracasei* strain was successfully isolated and differentiated from yogurt using morphologic, biochemical, and proteomic identification techniques, including MALDI-TOF MS. The preparation of cell-free supernatants confirmed the strain's ability to produce bioactive postbiotics by showing a strong antibacterial effect against major bacterial pathogens, including *E. coli*, *S. bovis*, *S. enterica*, and *E. faecalis*. The dose-dependent and similar impact of the antimicrobial effects to those of conventional antibiotics highlights the opportunity of these postbiotics as natural antibacterial agents.

In addition, these postbiotics have very little toxicity, preserving good cell viability, and Vero cell line cytotoxicity suggests, as per cytotoxicity tests, their safety for potential diet and medical use. These results add reliability to the growing body of research showing that, compared to probiotics, postbiotics can be used as a safe and efficient option for traditional antimicrobials, which have additional benefits such as stability and simplicity.

Overall, this study highlights the potential of postbiotics in infection prevention and the creation of functional foods, and it cautions against delaying more research into their mode of action and clinical effectiveness.

## ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to the Department of Microbiology, Dr. N.G.P. Arts and Science College, Coimbatore, for providing me with the necessary facilities and support to carry out this research work successfully.

## REFERENCES

**Aghebati-Maleki L, Hasannezhad P, Abbasi A, Khani N.** 2021. Antibacterial, antiviral, antioxidant, and anticancer activities of postbiotics: A review of mechanisms and therapeutic perspectives. Biointerface Research in Applied Chemistry **12**(2), 2629–2645. https://doi.org/10.33263/BRIAC122.26292645.

**Ammerman NC, Beier-Sexton M, Azad AF.** 2008. Growth and maintenance of Vero cell lines. Current Protocols in Microbiology **11**(1), A-4E. https://doi.org/10.1002/9780471729259.mca04es11.

**Ariful H, Saiful H, Dipa R, Zohorul IM.** 2023. Screening, isolation and evaluation of probiotic potential *Lactobacillus acidophilus* strains from available sources in Bangladesh. International Journal of Biosciences **23**(1), 68–80. http://dx.doi.org/10.12692/ijb/23.1.68-80.

**Bourebaba Y, Marycz K, Mularczyk M, Bourebaba L.** 2022. Postbiotics as potential new therapeutic agents for metabolic disorders management. Biomedicine & Pharmacotherapy **153**, 113138. https://doi.org/10.1016/j.biopha.2022.113138.

**Dahlan HA, Sani NA.** 2017. The interaction effect of mixing starter cultures on homemade natural yogurt's pH and viscosity. International Journal of Food Studies **6**(2), 152–158. https://doi.org/10.7455/ijfs/6.2.2017.a3.

**Elham N, Naheed M, Elahe M, Hossein MM, Majid T.** 2022. Selective cytotoxic effect of probiotic, paraprobiotic and postbiotics of *L. casei* strains against colorectal cancer cells: *In vitro* studies. Brazilian Journal of Pharmaceutical Sciences **58**, e19400. http://dx.doi.org/10.1590/s2175-97902022e19400.

**Hijová E.** 2024. Postbiotics as metabolites and their biotherapeutic potential. International Journal of Molecular Sciences **25**(10), 5441. https://doi.org/10.3390/ijms25105441.

**Kaewchomphunuch T, Charoenpichitnunt T, Thongbaiyai V, Ngamwongsatit N, Kaeoket K.** 2022. Cell-free culture supernatants of *Lactobacillus* spp. and *Pediococcus* spp. inhibit growth of pathogenic *Escherichia coli* isolated from pigs in Thailand. BMC Veterinary Research **18**(1), 60. https://doi.org/10.1186/s12917-022-03140-8.

**Karami S, Roayaei M, Hamzavi H, Bahmani M, Hassanzad-Azar H, Leila M, Rafieian-Kopaei M.** 2017. Isolation and identification of probiotic *Lactobacillus* from local dairy and evaluating their antagonistic effect on pathogens. International Journal of Pharmaceutical Investigation **7**(3), 137–141. https://dx.doi.org/10.4103/jphi.JPHI_8_17.

**Lee JY, Kim Y, Kim JI, Lee HY, Moon GS, Kang CH.** 2022. Improvements in human keratinocytes and antimicrobial effect mediated by cell-free supernatants derived from probiotics. Fermentation **8**(7), 332. https://doi.org/10.3390/fermentation8070332.

**Mani LE, Daniela AB, Aurelio LM.** 2022. The impacts of antimicrobial and antifungal activity of cell-free supernatants from lactic acid bacteria *in vitro* and foods. Comprehensive Reviews in Food Science and Food Safety **21**(1), 604–641. https://doi.org/10.1111/1541-4337.12872.

**Olson DW, Aryana KJ.** 2022. Probiotic incorporation into yogurt and various novel yogurt-based products. Applied Sciences **12**, 12607. https://doi.org/10.3390/app122412607.

**Ozbey G, Cambay Z, Yilmaz S, Aytekin O, Zigo F, Ozçelik M, Otlu B.** 2022. Identification of bacterial species in milk by MALDI-TOF and assessment of some oxidant-antioxidant parameters in blood and milk from cows with different health status of the udder. Polish Journal of Veterinary Sciences **25**(2), 269–277. https://doi.org/10.24425/pjvs.2022.141811.

**Phani Kumari YV, Kavita W.** 2024. Isolation and identification of probiotic bacteria from natural Neera to extend the shelf life of fresh fruits and vegetables. International Journal of Biosciences **24**(5), 25–37. http://dx.doi.org/10.12692/ijb/24.5.25-37.

**Piaru SP, Mahmud R, Perumal S.** 2012. Determination of antibacterial activity of essential oil of *Myristica fragrans* Houtt. using tetrazolium microplate assay and its cytotoxic activity against Vero cell line. International Journal of Pharmacology **8**(6), 572–576. https://doi.org/10.3923/ijp.2012.572.576.

**Thorakkattu P, Khanashyam AC, Shah K, Babu KS, Mundanat AS, Deliephan A, Deokar GS, Santivarangkna C, Nirmal NP.** 2022. Postbiotics: Current trends in food and pharmaceutical industry. Foods **11**(19), 3094. https://doi.org/10.3390/foods11193094.

**Widiastuti NK, Virginia NM, Yastawan I, Permatasari AAAP, Wiradana PA, Widhiantara IG, Sucipto TH.** 2023. Cytotoxicity evaluation of *Erythrina lithosperma* Miq. leaf extract against Vero cell lines: *In vitro* study. Research Journal of Pharmacy and Technology **16**(1), 153–158. http://dx.doi.org/10.52711/0974-360X.2023.00028.

**Yang KM, Kim JS, Kim HS, Kim YY, Oh JK, Jung HW, Park DS, Bae KH.** 2021. *Lactobacillus reuteri* AN417 cell-free culture supernatant as a novel antibacterial agent targeting oral pathogenic bacteria. Scientific Reports **11**(1), 1631. https://doi.org/10.1038/s41598-020-80921-x.

**Zendeboodi F, Khorshidian N, Mortazavian AM, DA Cruz AG.** 2020. Probiotic: Conceptualization from a new approach. Current Opinion in Food Science **32**, 103–123. https://doi.org/10.1016/j.cofs.2020.03.009.