Evaluation of antagonistic effect paraprobiotics obtained from traditional buttermilk 'Seet' on human pathogens

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Objective: This study aimed to evaluate the antagonistic effect of lactobacilli, commonly found in buttermilk, against four standard strains of pathogens - Staphylococcus aureus, Klebsiella pneumoniae, E. coli, Helicobacter pylori. The results showed that lactobacilli have the potential to prevent the development of a wide range of pathogens in both humans and animals(Huy et al., 2023).

Materials and Methods: Twenty-four bacterial isolates from seet (prepared from buffalo's milk collected from 8 different dairies, in Hisar) were characterized morphologically, culturally, and biochemically, and 13 isolates of them were found to be Lactobacillus spp, belonging to 7 different species. The results of the isolates tested for catalase, oxidase, urease, nitrate reduction, MRVP, Oxidative and Fermentative, and Caesin Hydrolysis. All the strains exhibited negative tests for catalase, oxidase, urease, and nitrate reduction but showed positive fermentative and casein hydrolysis tests. We then used the antimicrobial disc diffusion method to test the samples against four pathogens: Staphylococcus aureus (ATCC-6538), Klebsiella pneumoniae (K36), E. coli (ATCC 29181), Helicobacter pylori (ATCC 43579). The effectiveness of the isolates was evaluated through a disc diffusion test on Mueller-Hinton agar medium(Zhu et al., 2023).

Results: The results obtained indicated that out of 7 species the lactobacilli strains isolated from local dairy samples, only 3 had inhibitory effects on the studied pathogens. These strains were identified as L. acidophilus, L. casei, and L. delbrueckii. All three showed moderate activity, with the exception of L. casei, and L. delbrueckii, exhibited strong activity against E. coli and H. pylori, respectively, with an inhibition zone greater than 14 mm(Wang et al., 2022).

Conclusion: The present study suggested that various types of food, pharmaceutical products, and functional foods can be produced using these bacteria(Hussain et al., 2022).

Keywords: Antagonistic activity, Buttermilk, functional food, Lactobacillus and Paraprobiotics

INTRODUCTION

Paraprobiotics are metabolites of living organisms that can be added to food for their positive effects on the body's microbiome (Aggarwal et al., 2022). Lactic acid bacteria (LAB) are commonly used as protective cultures and are considered safe due to their specific characteristics (Fan et al., 2021). LAB includes genera such as Leuconostoc, Enterococcus, Lactobacillus, Lactococcus, Bifidobacterium, Pediococcus, and Streptococcus, which can reduce the population of harmful pathogens and promote the growth of beneficial microorganisms in the gastrointestinal tract (Kothari et al., 2019). LAB can also produce particular substances that help prevent the growth of pathogenic microorganisms (Savadogo et al, 2006). Scientific reports suggest that paraprobiotics have antiallergic and anticancer effects, can increase fat loss and immune response, and improve symptoms of digestive issues like irritable bowel syndrome and antibiotic-induced diarrhoea. Paraprobiotics are now used not only as a growth driver but also as a stimulator of the immune system and for disease prevention (Borges et al., 2021). Lactobacilli are important paraprobiotics that can be found in the mouth, intestine, and female genital tract (Qin et al., 2022). They produce antimicrobial metabolites that can help control undesirable microflora in the gut and can be used as natural bio-preservatives (Jordan et al., 2014; Monika et al., 2021). LABs are resistant to various digestive processes and can produce antimicrobial compounds that inhibit pathogenic microorganisms (Ibrahim et al., 2021, Batiha et al., 2021). These compounds may affect the metabolism or toxins of pathogenic bacteria. LAB can also protect dairy products from harmful bacteria and has been found to have antibacterial effects against various strains such as Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus (Vieco-Saize et al., 2019). A study was conducted to evaluate the antagonistic effect of isolated lactobacilli from Seet products against four standard strains of Staphylococcus aureus (ATCC-6538), Klebsiella pneumoniae (K36), E. coli (ATCC 29181), Helicobacter pylori (ATCC 43579).

MATERIALS AND METHODS

We collected buffalo milk samples from dairy farms in the neighbourhood of Hisar. Each sample was gathered in a sterile 250 mL vial and kept refrigerated (at 4^{o}C) during the delivery to the lab within 36 hours. The pH level of the buffalo milk was initially 6.8. To inoculate it, the milk was heated and then cooled to 40°C. Seet

was prepared (Saini and Kaur, 2022). We characterized 24 bacterial isolates from seet. We conducted morphological, cultural, and biochemical tests and found that 13 isolates of them were Lactobacillus spp (Saini and Kaur, 2022). We tested the isolates for catalase, oxidase, urease, nitrate reduction, MRVP, oxidative and fermentative, and casein hydrolysis. All the strains showed negative test results for catalase, oxidase, urease, and nitrate reduction, but gram-positive tested positive for fermentative and casein hydrolysis. (Saini and Kaur, 2022).

Isolation and Identification of Lactobacillus (lactic acid bacteria)

In this experiment, a flask containing MRS Broth was used as an enrichment media. Two grams of each sample were transferred to the flask, and distilled water was added to make the total volume 100 ml. The mixture was then incubated at 37°C for 24 hours. After incubation, 100 µl of the enriched samples were spread on MRS agar and incubated at 37°C under anaerobic conditions for 48 hours. Bacterial colonies were purified through subsequent subcultures. To identify the bacteria, classic microbiology tests including Gram staining, catalase and oxidase tests, motility, and carbohydrate fermentation tests were performed. All Gram-positive and catalase-negative bacilli were selected for the assessment of antimicrobial ability. (Saini and Kaur, 2022)

To evaluate the antimicrobial effect of the isolates, a disc diffusion test was conducted on an MHA medium plated with three pathogens. Fresh cultures of the isolates were centrifuged (8000 rpm, 15 min), and the supernatants were removed. Blank discs were inoculated with 40 µl supernatant of each isolate and were placed on separate MHA medium inoculated with Staphylococcus aureus (ATCC-6538), Klebsiella pneumoniae (K36), E. coli (ATCC 29181), Helicobacter pylori (ATCC 43579)**.** After incubation of all agar media at 37°C for 24 hours, the growth inhibition zones of pathogens and isolated lactobacilli inhibitory ability were assessed. (Karami et al., 2017)

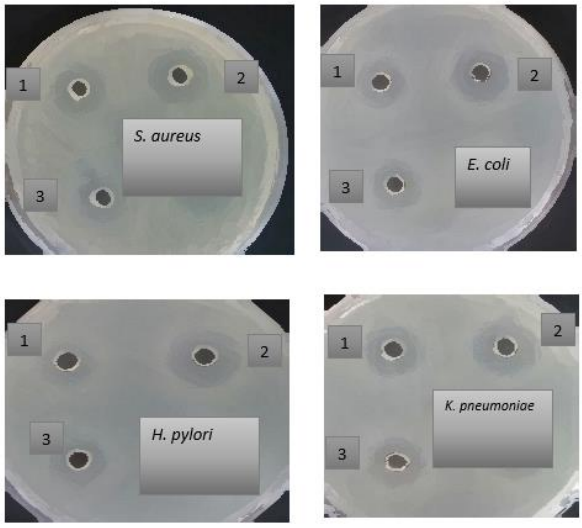
RESULT AND DISCUSSION

Out of 13 lactobacillus isolates total genera obtained were 7 (L. delbrueckii, L. helveticus, L. bulgaricus, L. casei, L. brevis, L. acidophilus, L. paracasei) (Saini and Kaur, 2022). According to the biochemical studies as reported in previous paper, all of them showed negative catalase, oxidase, urease, citrate, and nitrogenase activity. All were non-motile and gram positive. (Saini and Kaur, 2022). In the present study obtained results showed that only 3 species of isolated lactobacilli from seet had inhibitory effects on understudy pathogens including L. acidophilus, L. casei, and L. delbrueckii(Jarlöv et al., 2022).

Table 1: Growth inhibition zone diameter (mm) of pathogens with three Lactobacillus strains

SPECIES IDENTIFIED	**Pathogen**			
	Staphylococcus aureus (ATCC-6538)	**Klebsiella pneumoniae (K36)**	**E. coli (ATCC 29181)**	**Helicobacter pylori (ATCC 43579)**
Lactobacillus acidophilus	7.0	7.0	13.8	18.2
Lactobacillus casei	15.0	10.8	15.7	16.4
Lactobacillus delbrueckii	12.5	9.0	13.5	14.0

Figure 1: Antagonistic activity of Lactobacillus strains against test pathogens



Dairy products particularly have long been acknowledged as beneficial to human health (Barros et al., 2020; Siciliano et al., 2021). The antagonistic effects of LAB and lactobacilli against different diseases have been studied in recent years by numerous scientists who have isolated and identified these microorganisms from traditional goods all over the world (Hassan et al., 2020; Zapaśnik et al., 2022). Numerous bacteria, including lactobacilli, are capable of eradicating infections through a variety of ways, including competitive elimination, which ensures the safety of food Castellano et al., 2017).

Scientists have been attempting to replace synthetic medications with natural alternatives for a very long time (Boman et al., 2003). To prevent or treat diseases, several natural products and techniques are now used. One of these techniques is the use of paraprobiotics (de Almada et al., 2016). Normal intestinal flora such as lactobacilli and bifidobacteria play a crucial role in human health by avoiding colon cancer, decreasing cholesterol, activating the immune system, and preventing intestinal infections (Eslami et a., 2019). Lactic acid and other organic acids are produced by probiotic bacteria, which also work to lower the pH of the environment and stop the growth of numerous bacteria (Zhang et al., 2015). These bacteria generate natural preservatives called bacteriocin and other antibacterial substances. The metabolites produced by these bacteria and separated by centrifugation in this investigation were discovered to have the ability to inhibit the growth of harmful bacteria (Masood et al., 2011).

Antibiotic resistance has been rising in recent years. Paraprobiotics have been found to have positive benefits on the prevention and treatment of various diseases. Probiotics might slow the growth of H. pylori, according to numerous researches. Paraprobiotic bifidobacteria may also produce bacteriocins that are active against H. pylori, although Lactobacillus has received much of the attention in this regard. Paraprobiotics lessen the negative effects of H. pylori regimens and might marginally improve the effectiveness of eradication. Some Balkan populations' longevity has been correlated with their custom of consuming yogurt. Additionally, probiotics are said to have anticarcinogenic properties. (Mladenova-Hristova, 2013).

Furthermore, consuming the bacteria Lactobacillus casei, Lactobacillus acidophilus, from culture supernatants had inhibitory effects on a variety of disease-causing microorganisms, according to Gillor et al, 2008. In addition to this, a study by Fernández et al., 2003 have shown S. aureus had the most ability to be inhibited from growing, along with E. coli, H. pylori, by the lactobacilli strains isolated from dairy products(Zeidan et al., 2022).

In the current research work we found that L. acidophilus showed maximum zone of inhibition of around 18mm against K. pneumoniae. S. aureus was maximally inhibited by L. casei exhibiting inhibition zone of 15mm(Mao et al., 2022). The present findings were consistent with the studies conducted previously. Some lactic acid bacterial strains were isolated and characterized from human milk, infant feces, and fermented grapes and dates, as potential probiotics with antimicrobial activity against some human pathogenic strains. The results showed that all the isolated Lactobacillus strains, exhibited strong inhibition on the growth of Staphylococcus sp., and the L. casei strains showed strong antagonistic activities against Helicobacter pylori and good inhibition against Staphylococcus aureus. (Shokryazdan et al., 2014)

Previous studies have indicated that L. delbrueckii had a good inhibitory effect on the E. coli growth (Abedi et al., 2013). In another study, Cells of Lactobacillus delbrueckii subsp. lactis RM2-5 were added to various meat model systems that had been inoculated with Escherichia coli O157:H7. Experiments in which L. delbrueckii subsp. lactis RM2-5 was directly applied to the surfaces of beefsteaks resulted in significant ($P < 0.05$) reductions in the growth of psychrotrophs and coliforms plus a slight decrease in the numbers of E. coli O157:H7 (Senne et al., 2003). One more previous study confirmed that lactic acid levels from Lactobacillus delbrueckii subsp. exhibited antimicrobial activity against Helicobacter pylori ATCC 43504. It was observed that inhibitory activities of only ten strains were due to bacteriocin-like substances (Aslim et al., 2011).

Recent studies have also published a research work indicating antagonistic properties of Lactobacillus delbrueckii 45E (Ld45E) against several species of bacteria, namely, Group B Streptococcus (GBS), Escherichia coli, Klebsiella spp., and Candida parapsilosis, as well as to determine the concentration of interleukin-17 (IL-17) in the presence of Ld45E (Bnfaga et al., 2023). In our present research work also, L. delbrueckii has shown maximum inhibitory zone of around 14mm, indicating relatively good antagonistic activity against pathogens(Kim et al., 2022).

According to the findings of the current investigation, L. caesi had the largest impact on H. pylori, and E. coli. H. pylori was most impacted by L.delbrueckii . Except for L. acidophillus the other two spesies L. Casei and L. delbrueckii had comparatively significant activity (inhibition zone >=14 mm) against understudy pathogens. Thus above findings suggested that microbiota from seet, a traditional buttermilk, possess potential antagonistic activity against human pathogens(Biasetti et al., 2022).

CONCLUSION

Based on the findings of this study, food preservation and human health are significantly impacted by the antagonistic effects of chemicals produced by bacteria on a variety of microorganisms. For the manufacturing of various culinary and pharmaceutical goods, these microorganisms can be grown. Additionally, they can be employed in the creation of novel functional meals. As a result, it is advised to utilize more dairy products that contain probiotics and to identify and produce foods that have the greatest and most potent levels of lactobacilli.

Conflicts of interest

There are no conflicts of interest.

References

Abedi, D., Feizizadeh, S., Akbari, V., & Jafarian-Dehkordi, A. (2013). In vitro anti-bacterial and anti-adherence effects of Lactobacillus delbrueckii subsp bulgaricus on Escherichia coli. Research in pharmaceutical sciences, 8(4), 260.

Aggarwal, N., Kitano, S., Puah, G. R. Y., Kittelmann, S., Hwang, I. Y., & Chang, M. W. (2022). Microbiome and human health: Current understanding, engineering, and enabling technologies. Chemical Reviews, 123(1), 31-72.

Aslim, B., Onbasili, D., & ZN, Y. (2011). Determination of Lactic Acid Production and Antagonistic Activity against Helicobacter pylori of L. delbrueckii subsp. bulgaricus and S. thermophilus Strains. Kafkas Üniversitesi Veteriner FakÜltesi Dergisi, 17(4).

Batiha, G. E. S., Hussein, D. E., Algammal, A. M., George, T. T., Jeandet, P., Al-Snafi, A. E., ... & Cruz-Martins, N. (2021). Application of natural antimicrobials in food preservation: Recent views. Food Control, 126, 108066.

Barros, C. P., Guimaraes, J. T., Esmerino, E. A., Duarte, M. C. K., Silva, M. C., Silva, R., ... & Cruz, A. G. (2020). Paraprobiotics and postbiotics: Concepts and potential applications in dairy products. Current opinion in food science, 32, 1-8.

Bnfaga, A. A., Lee, K. W., Than, L. T. L., & Amin-Nordin, S. (2023). Antimicrobial and immunoregulatory effects of Lactobacillus delbrueckii 45E against genitourinary pathogens. Journal of Biomedical Science, 30(1), 19.

Boman, H. G. (2003). Antibacterial peptides: basic facts and emerging concepts. Journal of internal medicine, 254(3), 197-215.

Borges, N., Keller-Costa, T., Sanches-Fernandes, G. M., Louvado, A., Gomes, N. C., & Costa, R. (2021). Bacteriome structure, function, and probiotics in fish larviculture: the good, the bad, and the gaps. Annual Review of Animal Biosciences, 9, 423-452.

Castellano, P., Pérez Ibarreche, M., Blanco Massani, M., Fontana, C., & Vignolo, G. M. (2017). Strategies for pathogen biocontrol using lactic acid bacteria and their metabolites: A focus on meat ecosystems and industrial environments. Microorganisms, 5(3), 38.

de Almada, C. N., Almada, C. N., Martinez, R. C., & Sant'Ana, A. S. (2016). Paraprobiotics: Evidences on their ability to modify biological responses, inactivation methods and perspectives on their application in foods. Trends in food science & technology, 58, 96-114.

Eslami, M., Yousefi, B., Kokhaei, P., Hemati, M., Nejad, Z. R., Arabkari, V., & Namdar, A. (2019). Importance of probiotics in the prevention and treatment of colorectal cancer. Journal of cellular physiology, 234(10), 17127-17143.

Fan, Y., & Pedersen, O. (2021). Gut microbiota in human metabolic health and disease. Nature Reviews Microbiology, 19(1), 55-71.

Fernández, M. F., Boris, S., & Barbes, C. (2003). Probiotic properties of human lactobacilli strains to be used in the gastrointestinal tract. Journal of applied microbiology, 94(3), 449-455.

Gillor, O., Etzion, A., & Riley, M. A. (2008). The dual role of bacteriocins as anti-and probiotics. Applied microbiology and biotechnology, 81, 591-606.

Hassan, M. U., Nayab, H., Rehman, T. U., Williamson, M. P., Haq, K. U., Shafi, N., & Shafique, F. (2020). Characterisation of bacteriocins produced by Lactobacillus spp. isolated from the traditional Pakistani yoghurt and their antimicrobial activity against common foodborne pathogens. BioMed research international, 2020.

Ibrahim, S. A., Ayivi, R. D., Zimmerman, T., Siddiqui, S. A., Altemimi, A. B., Fidan, H., ... & Bakhshayesh, R. V. (2021). Lactic acid bacteria as antimicrobial agents: Food safety and microbial food spoilage prevention. Foods, 10(12), 3131.

Jordan, K., Dalmasso, M., Zentek, J., Mader, A., Bruggeman, G., Wallace, J., ... & Malakauskas, M. (2014). Microbes versus microbes: control of pathogens in the food chain. Journal of the Science of Food and Agriculture, 94(15), 3079-3089.

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.

Kothari, D., Patel, S., & Kim, S. K. (2019). Probiotic supplements might not be universally-effective and safe: A review. Biomedicine & Pharmacotherapy, 111, 537-547.

Monika, K., Malik, T., Gehlot, R., Rekha, K., Kumari, A., Sindhu, R., & Rohilla, P. (2021). Antimicrobial property of probiotics. Environment Conservation Journal, 22(SE), 33-48.

Masood, M. I., Qadir, M. I., Shirazi, J. H., & Khan, I. U. (2011). Beneficial effects of lactic acid bacteria on human beings. Critical reviews in microbiology, 37(1), 91-98.

Mladenova-Hristova, I. (2013). The inhibitory effect of genus Lactobacillus on Helicobacter pylori infection. Trakia J Sci, 4, 299-303.

Qin, D., Ma, Y., Wang, Y., Hou, X., & Yu, L. (2022). Contribution of Lactobacilli on Intestinal Mucosal Barrier and Diseases: Perspectives and Challenges of Lactobacillus casei. Life, 12(11), 1910.

Savadogo, A., Ouattara, A. C., Bassole, H. I., & Traore, S. A. (2006). Bacteriocins and lactic acid bacteria-a minireview. African journal of biotechnology, 5(9).

Senne, M. M., & Gilliland, S. E. (2003). Antagonistic action of cells of Lactobacillus delbrueckii subsp. lactis against pathogenic and spoilage microorganisms in fresh meat systems. Journal of food protection, 66(3), 418-425.

Shalini Saini*, Harpreet Kaur. Isolation and Biochemical Characterization of Lactic Acid Bacteria from "Seet", a Traditional Buttermilk . International Journal of Food and Nutritional Sciences, 11(12), 7886-7906.

Shokryazdan, P., Sieo, C. C., Kalavathy, R., Liang, J. B., Alitheen, N. B., Faseleh Jahromi, M., & Ho, Y. W. (2014). Probiotic potential of Lactobacillus strains with antimicrobial activity against some human pathogenic strains. BioMed research international, 2014.

Siciliano, R. A., Reale, A., Mazzeo, M. F., Morandi, S., Silvetti, T., & Brasca, M. (2021). Paraprobiotics: A new perspective for functional foods and nutraceuticals. Nutrients, 13(4), 1225.

Vieco-Saiz, N., Belguesmia, Y., Raspoet, R., Auclair, E., Gancel, F., Kempf, I., & Drider, D. (2019). Benefits and inputs from lactic acid bacteria and their bacteriocins as alternatives to antibiotic growth promoters during food-animal production. Frontiers in microbiology, 10, 57.

Zapaśnik, A., Sokołowska, B., & Bryła, M. (2022). Role of lactic acid bacteria in food preservation and safety. Foods, 11(9), 1283.

Zhang, Y. J., Li, S., Gan, R. Y., Zhou, T., Xu, D. P., & Li, H. B. (2015). Impacts of gut bacteria on human health and diseases. International journal of molecular sciences, 16(4), 7493-7519.

Huy, D. Q., Sadjoli, N., Azam, A. B., Elhadidi, B., Cai, Y., & Seet, G. (2023). Object perception in underwater environments: A survey on sensors and sensing methodologies. Ocean Engineering, 267, 113202.

Zhu, Z., Hu, Z., Seet, H. L., Liu, T., Liao, W., Ramamurty, U., & Nai, S. M. L. (2023). Recent progress on the additive manufacturing of aluminum alloys and aluminum matrix composites: Microstructure, properties, and applications. International Journal of Machine Tools and Manufacture, 104047.

Wang, Z., McWilliams-Koeppen, H. P., Reza, H., Ostberg, J. R., Chen, W., Wang, X., ... & Brown, C. E. (2022). 3D-organoid culture supports differentiation of human CAR+ iPSCs into highly functional CAR T cells. Cell Stem Cell, 29(4), 515-527.

Hussain, S., Seet, P. S., Ryan, M., Iranmanesh, M., Cripps, H., & Salam, A. (2022). Determinants of switching intention in the electricity markets-An integrated structural model approach. Journal of Retailing and Consumer Services, 69, 103094.

Jarlöv, A., Ji, W., Zhu, Z., Tian, Y., Babicheva, R., An, R., ... & Zhou, K. (2022). Molecular dynamics study on the strengthening mechanisms of Cr–Fe–Co–Ni high-entropy alloys based on the generalized stacking fault energy. Journal of Alloys and Compounds, 905, 164137.

Zeidan, A. M., DeAngelo, D. J., Palmer, J., Seet, C. S., Tallman, M. S., Wei, X., ... & Stock, W. (2022). Phase 1 study of anti-CD47 monoclonal antibody CC-90002 in patients with relapsed/refractory acute myeloid leukemia and high-risk myelodysplastic syndromes. Annals of hematology, 101(3), 557-569.

Mao, Z., Nesterenko, P. A., McLaughlin, J., Deng, W., Burton Sojo, G., Cheng, D., ... & Witte, O. N. (2022). Physical and in silico immunopeptidomic profiling of a cancer antigen prostatic acid phosphatase reveals targets enabling TCR isolation. Proceedings of the National Academy of Sciences, 119(31), e2203410119.

Kim, J. T., Zhang, T. H., Carmona, C., Lee, B., Seet, C. S., Kostelny, M., ... & Zack, J. A. (2022). Latency reversal plus natural killer cells diminish HIV reservoir in vivo. Nature communications, 13(1), 121.

Biasetti, P., Hildebrandt, T. B., Göritz, F., Hermes, R., Holtze, S., Galli, C., ... & de Mori, B. (2022). Ethical analysis of the application of assisted reproduction technologies in biodiversity conservation and the case of white rhinoceros (Ceratotherium simum) ovum pick-up procedures. Frontiers in Veterinary Science, 9, 831675.