Environment and Ecology 41 (4A) : 2526—2531, October—December 2023
Article DOI: https://doi.org/10.60151/envec/BBSS8618
ISSN 0970-0420

Effectiveness of Anaerobic Probiotic Bacterium *Bifidobacterium bifidum* against Food Spoilage Bacteria

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Received 22 May 2023, Accepted 7 August 2023, Published on 31 October 2023

ABSTRACT

The efficiency of an anaerobic probiotic bacterium against bacteria that cause food deterioration was tested in the current investigation. Based on our prior research, *Bifidobacterium bifidum* was chosen as an anaerobic probiotic bacterium. By using the conventional method of isolation, food spoilage bacteria were isolated, and through biochemical and molecular characterization, isolates of *Escherichia coli, Staphylococcus aureus, Shigella dysenteriae,* and *Salmonella typhi* were detected. *Bifidobacterium bifidum* demonstrated strong inhibitory capacity against all identified food spoilage bacteria in the agar well diffusion method, with the exception of *Shigella dysenteriae.*

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Keywords *Bifidobacterium bifidum,* Bio-preservation, Food spoilage, Probiotic.

INTRODUCTION

Foods are essentially organic compounds (both plant- and animal-derived) that are consumed for nourishment. Food can become spoiled by microbes because it contains moisture, protein, lipids, carbohydrates, minerals, and other organic compounds. The term "microbial spoiling" refers to food deterioration caused by microbes. Additionally, it is the primary contributor to foodborne illnesses (Tianli *et al.* 2014). Food safety is intimately correlated with food spoilage, which is the process of decreasing food edibility (Steele 2004). *Salmonella typhi, Listeria monocytogenes, Staphylococcus aureus, Bacillus subtilis, Shigella dysenteriae, Escherichia coli* O157: H7, and *Candida* spp. are only a few of the pathogenic bacteria that have been identified as causative agents for food spoilage and foodborne diseases (Sokmen *et al.* 2004 He *et al.* 2010). Food spoilage is a major global public health concern because it leads to serious foodborne intoxication and significant financial losses for the food-producing and processing industry.

Food deterioration can be detected by changes in color, flavor, odor, and texture (Rahman 2007). Consumers and the food business now place a greater emphasis on the microbiological safety of food.

Foods need to be preserved in order to keep their quality for a longer time. Increased shelf life while preserving original food quality is the main goal of food preservation. Food can be preserved using a variety of techniques, including physical, chemical, and biological techniques. Consumers today favor foods with little to no processing and no added chemicals. The most dependable and beneficial way of food preservation to meet these requirements is bio-preservation. Utilizing microorganisms or their metabolites, bio-preservation refers to extending the shelf life and improving the quality and safety of food (Ross *et al.* 2002). Due to the probiotic bacteria's antibacterial properties against foodborne pathogens and spoilage microorganisms, interest in using it as a bio-preservative has increased (Kim *et al.* 2022, Choeisoongnern *et al.* 2021, Saud *et al.* 2020, Hossain *et al.* 2018 and Fang *et al.* 2018). The most prevalent and well-known members of the intestinal microflora included in the probiotics category are *Bifidobacteria bifidum* (Espirito *et al.* 2003, Nielsen *et al.* 2003). These are naturally occurring bacteria that are Generally Recognized as Safe (GRAS) due to their lack of human and animal pathogenicity (Patil *et al.* 2010). According to Nielsen *et al.* (2003) and Zacarías *et al.* (2020), these microbes are anaerobic, nutritionally fastidious, gram-positive, non-spore-forming, pleomorphic rod, catalase-negative, and have a high G+C content.

In our earlier research (Raisagar and Shukla 2022), *Bifidobacterium bifidum* showed tolerance to bile salt, phenol, and NaCl concentrations as well as growth in a variety of pH and temperature ranges. Consequently, it possesses the ideal probiotic qualities. The antibacterial activity of *Bifidobacterium bifidum* was assessed in the current study to determine its efficacy against bacteria that cause food spoilage.

MATERIALS AND METHODS

Collection of samples

Samples of spoiled food and dairy products were gathered at Prayagraj local market in Uttar Pradesh, India. All of the samples were transferred to the lab in refrigerated condition after being individually wrapped in sterile dry polyethylene zip closure bags (Fig. 1).

Fig. 1. Sample collection in zip closer bags.

Isolation of food spoilage bacteria

The conventional method of isolation was used to isolate the bacteria that cause food deterioration. The samples were first divided into small pieces using a sterilized knife and placed in sterile plastic bags with 9 ml of a sterile dilution blank (Ringer's solution). Rinsate was extracted by shaking the bags vigorously. The rinse was then serially diluted up to five times before being pour-plated into specific media. For 24 to 48 hours, all of the plates were incubated at a temperature of 37°C. This process was conducted separately for each sample. Isolates were chosen based on cultural characteristics, and pure cultures were kept under refrigeration at 4°C for later use.

Biochemical characterization of isolates

After cultural characterization, all of the isolates were biochemically characterized. Catalase, Oxidase, Indole, Methyl Red, Voges Proskauer, Citrate, Nitrate reduction, Urease, and Carbohydrate Fermentation Tests with Sugars Arabinose, Fructose, Glucose, Lactose, Maltose, Mannitol, Raffinose, Sucrose, Xylose, and Sorbitol were used to characterize the biochemical makeup of the isolates The. tests were all conducted according to the protocol (Cappuccino and Sherman 2005).

Molecular characterization of isolates

The isolates were molecularly characterized at Scan-

gene Labs Pvt Ltd, Delhi using the Sanger technique. This approach included the following steps: Genomic DNA separation, agarose gel electrophoresis, genomic DNA quantification, partial 16srRNA PCR amplification, gel purification of the PCR amplified product, and automated DNA clone sequencing.

Procurement, revival and maintenance of anaerobic probiotic bacteria

Bifidobacterium bifidum, the selected probiotic bacteria, was procured from the National Collection of Industrial Microorganisms (NCIM), Pune in dried culture form. MRS (De Man Rogosa Sharpe) agar slants supplemented with 0.05% L-cysteine hydrochloride monohydrate were used to revive *Bifidobacterium bifidum* under anaerobic conditions at 37°C for 24 hours. The candle jar method (Saha *et al.* 2016) was employed to maintain anaerobic conditions. The MRS agar slant inoculated with *Bifidobacterium bifidum,* a lit candle, iron wool treated with acidified copper sulfate and a strip of methylene blue were stored inside a desiccator unit in this procedure. Wax was used to seal the desiccator unit's lid tightly. The lit candle burned the oxygen, releasing CO_2 in the process. Iron wool absorbed any remaining oxygen. In this situation, methylene blue serves as a sign of anaerobic conditions; in the absence of oxygen, it is white or colorless. After that, the desiccator unit was housed in an incubator. Cultures were stored at 4°C and sub-cultured in the same media slants and incubation conditions at regular intervals of 10 to 15 days after being incubated at 37°C for 24 hrs.

Antibacterial activity against food spoilage bacteria

The agar well diffusion method was used to assess the anaerobic probiotic culture *Bifidobacterium bifidum's* antibacterial activity against isolates (Abdel-Raouf *et al*. 2014). The food spoilage bacteria were pour-plated onto Muller-Hinton agar (MHA) using just one strain as an indication. With the aid of a sterile cork borer, a 6 mm well was created in the MHA plate, which was then filled with a 24-hr-old broth culture of *Bifidobacterium bifidum.* For 24 hrs, all of the plates were incubated at 37°C. The inhibition zone was measured after incubation.

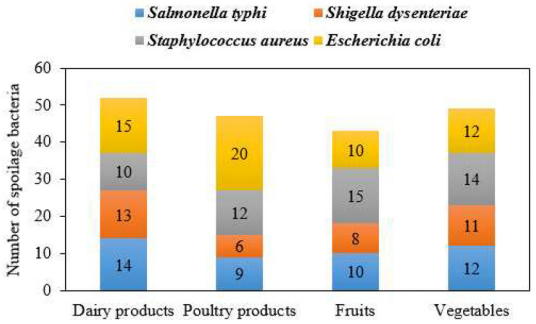

Fig. 2. Occurrence of food spoilage bacteria.

RESULTS

Isolation of food spoilage bacteria

191 food spoilage bacteria were found in selected samples. The majority of spoilage bacteria, 52, were found in dairy samples, followed by vegetable samples with 49 isolates and chicken samples with 47 isolates. Fruit samples revealed 43 isolates of the lowest spoilage bacteria (Fig. 2).

Identification of food spoilage bacterial isolates

All of the isolates tested positive for the catalase test, the nitrate reduction test, and the MR test during biochemical characterization. Except for S*higella dysenteriae,* all of the isolates failed the oxidase test, and only *Staphylococcus aureus* passed the VP, urease, and citrate tests. Each and every *Escherichia coli* isolate tested positive for indole (Fig. 3). All isolates, with the exception of *Escherichia coli*, were fructose positive and arabinose negative during the sugar fermentation test. All the isolates were able to ferment glucose (except *Salmonella typhi*) and lactose and mannitol (except *Shigella dysenteriae)* and sorbitol (except *Staphylococcus aureus*). *Salmonella typhi* and *Staphylococcus aureus* were both maltose fermenters. While *Staphylococcus aureus* and *Escherichia coli* displayed sucrose fermentation. *Salmonella typhi* and *Escherichia coli* were the only bacteria found to ferment xylose. The isolates didn't exhibit raffinose fermentation at all. *Escherichia coli, Staphylococcus aureus, Salmonella typhi,* and *Shigella dysenteriae* were identified in selected samples through molecular characterization using 16S rRNA sequencing,

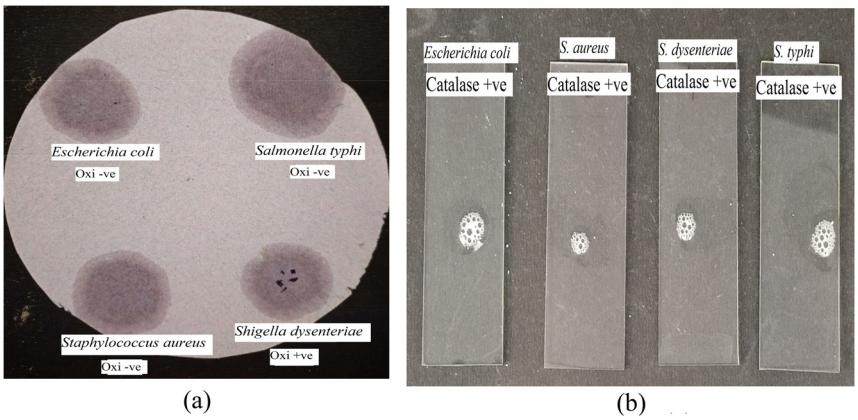

Fig. 3. Biochemical characterization of isolates (a) Oxidase test, (b) Catalase test, (c) Indole test.

electropherograms, and BLAST analysis.

Antibacterial activity against food spoilage bacteria

The anaerobic probiotic bacteria, *Bifidobacterium bifidum*, that was chosen in the agar well diffusion method, showed antimicrobial activity against isolated food spoilage bacteria, namely *Escherichia coli, Staphylococcus aureus, Shigella dysenteriae,* and *Salmonella typhi. Escherichia coli* had a reported zone of inhibition of 22 mm, *Staphylococcus aureus* of 30 mm, *Shigella dysenteriae* of 14 mm, and *Salmonella typhi* of 24 mm. Using the method proposed by Pisano *et al.* (2014), Carasi *et al*.(2014) the width of the clear zone, or R, was also computed after recording the zone of inhibition. This R-value was used to determine the level of inhibition. When R is between 2 and 5 mm and greater than 6 mm, the inhibition score is regarded as having a low inhibition capacity. *Bifidobacterium bifidum* has shown good inhibition capacity in the current investigation against *Escherichia coli* (R = 8 mm), *Staphylococcus aureus* (R = 12 mm), and *Salmonella typhi* (R = 9 mm), but *Shigella dysenteriae* (R = 4 mm) had low inhibition ability (Table 1).

DISCUSSION

Bifidobacterium bifidum, an specific anaerobic probiotic bacteria, demonstrated the largest zone of inhibition against *Staphylococcus aureus* and the lowest zone of inhibition against *Shigella dysenteriae* in the current investigation. Forhad *et al.* (2015) chose anaerobic *Bifidobacterium* coupled with *Lactobacillus casei, Lactobacillus fermentum,* and *Lactobacillus acidophilus* as probiotic culture, which is similar to the current investigation. They measured a 12 mm, 15 mm, and 10 mm zone of inhibition against *Escherichia coli*, *Salmonella* spp., and *Shigella* species, respectively when evaluating the antibacterial activ-

Table 1. Antibacterial activity of *Bifidobacterium bifidum* against food spoilage bacteria.

Parameters	Isolated food spoilage bacteria			
	Escherichia coli	*Staphylococcus aureus*	*Shigella dysenteriae*	*Salmonella typhi typhi*
Zone of inhibition (in mm)	22	30	14	24
Width of clear zone (R) (in mm)	08	12	04	09
Inhibition capacity	High	High	Low	High

R = 2 to 5 mm = low inhibition capacity and R > 6 = high inhibition capacity.

ity of *Bifidobacterium*. El-Jakee *et al.* (2010) also previously investigated the antibacterial activity of *Bifidobacterium bifidum* against *Salmonella* species and measured zone of inhibition was 8 mm. Alwan *et al.* (2014) also investigated the antibacterial activity of probiotic bacteria against *Staphylococcus aureus* and measured zone of inhibition was 15 mm. Probiotic *Lactobacillus plantarum* has been demonstrated in a 2015 study by Sridevi *et al*.(2015) to have antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. According to research by El-Jakee *et al*. (2010), *Lactobacillus acidophilus* and *Lactobacillus helveticus* had antibacterial activity against *Salmonella* species with an 11 mm zone of inhibition.

Probiotic strains of *Lactobacillus fermentum, Lactobacillus helveticus, Lactobacillus paracasei, Lactobacillus lactis,* and *Bifidobacterium longum* were chosen for a study by Gad *et al*.(2016) because of their antibacterial activity against the foodborne pathogens *Escherichia coli*, *Staphylococcus aureus,* and *Shigella* species. With the exception of *Lactobacillus lactis,* which exhibited no inhibition against any of the chosen food-borne pathogens, all of the probiotics that were chosen demonstrated antibacterial activity. *Bifidobacterium longum*, in contrast, did not inhibit *Staphylococcus aureus*. With the exception of *Shigella dysenteriae, Bifidobacterium bifidum* demonstrated strong inhibition against all identified food spoilage bacteria in the current investigation. This might be because different probiotic bacteria produce different metabolites.

CONCLUSION

According to the results of the current investigation, *Bifidobacterium bifidum,* a probiotic anaerobe, has antibacterial activity against isolated food spoilage bacteria. Consequently, it might be a compelling candidate for food bio-preservation, albeit more research is required to assess the structure and characteristics of the component that is in charge of the antibacterial activity of particular probiotic bacteria. Additionally, an *in-vivo* investigation is required to confirm the safety of employing *Bifidobacterium bifidum* in the food chain.

ACKNOWLEDGMENT

The authors would like to thank the Department of Dairy Microbiology, Warner College of Dairy Technology, SHUATS, Prayagraj, Uttar Pradesh, India for providing facilities to carry out the research.

REFERENCES

Abdel-Raouf M, Nabil M, El-Sayed M, Center GE (2014) Antimicrobial activities of some herbs extract on food borne bacteria. *J Am Sci* 10: 76-85.

Alwan AH, Alsakini A, Haider, Abdulrassak FN (2014) Original research article the protective effect of probiotics (*Lactobacillus acidophilus* and *Saccharomyces boulardii*) against infections caused by *Staphylococcus aureus in vitro* and *In vivo*. *Int J Curr Microbiol Appl Sci* 3(7): 886–890.

Cappuccino JG, Sherman N (2005) Microbiology: A laboratory manual 7th ed. Pearson Education. Inc and Darling Kindersley (India), pp 143- 203.

Carasi P, Diaz M, Racedo SM, Antoni GD, Urdaci MC (2014) Safety characterization and antimicrobial properties of kefir-isolated *Lactobacillus kefiri*. Biomed Res Int 208974: 1-7.

Choeisoongnern T, Sirilun S, Waditee-Sirisattha R, Pintha K, Peerajan S, Chaiyasut C (2021) Potential probiotic *Enterococcus faecium* OV3-6 and its bioactive peptide as alternative bio-preservation. *Foods* 10(10): 1–19.

El-Jakee J, Moussa IM, Nada SA, Mohamed KF, Ashgan MH, Mohamed ML (2010) Influence of probiotics mixture on *Salmonella typhimurium* in Mice. *Int J Microbiol Res* 1(2) : 50– 61.

Espirito Santo MLP, Beirao LH, Santanna ES, Dalcin EB, Franco BGM (2003) Bacteriocinogenic effect of *Lactobacillus sakei* 2a on microbiological quality of fermented *Sardinella brasil iensis*. *Brazilian Arch Biol Technol* 46: 553-561. https://doi.org/10.1590/s1516-89132003000400009

Fang F, Xu J, Li Q, Xia X, Du G (2018) Characterization of a *Lactobacillus brevis* strain with potential oral probiotic properties. *BMC Microbiol* 18(221): 1–9.

Forhad MH, Rahman SMK, Rahman S, Saikot FK, Biswas KC (2015) Probiotic properties analysis of isolated lactic acid bacteria from buffalo milk. *Arch Clinical Microbiol* 7 (1): 1-6.

Gad SA, El-baky RM A, Bakr A, Ahmed F, Fadl G (2016) *In vitro* evaluation of probiotic potential of five lactic acid bacteria and their antimicrobial activity against some enteric and food-borne pathogens. *Afr J Microbiol Res Full* 10(12): 400–409.

He FY, Yang G, Yang L, Yu (2010) Studies on antibacterial activity and antibacterial mechanism of a novel polysaccharide from *Streptomyces virginia* $H0_3$. *Food Control* 21: 1257–1262. https://doi.org/10.1016/j.foodcont.2010.02.013

Hossain N, Humayun S, Shabnam J, Rahman MB, Shamimma AM-M (2018) Probiotic properties of *Bifidobacterium* species isolated from mother's milk and infant feces. *Asian-Australasian J Biosci Biotechnol* 3(2): 122–135.

Kim JH, Lee ES, Song KJ, Kim BM, Ham JS, Oh MH (2022)

Development of desiccation-tolerant probiotic biofilms inhibitory for growth of foodborne pathogens on stainless steel surfaces. *Foods* 11(6): 831.

Nielsen DS, Moller PI, Rosenfeldt V, Paerregaard A, Michaelsen KF, Jakobsen M (2003) Case study of the distribution of mucosa-associated *Bifidobacterium* species, *Lactobacillus* species, and other lactic acid bacteria in the human colon. *Appl Environm Microbiol* 69 (12): 7545-7548.

Patil MM, Pal A, Anandand T, Ramana KV (2010) Isolation and characterization of lactic acid bacteria from curd and cucumber. *Ind J Biotechnol* 9: 166-172.

Pisano MB, Viale S, Conti S, Fadda M, Deplano M (2014) Preliminary evaluation of probiotic properties of *Lacto bacillus* strains isolated from Sardinian dairy products. *Biomed Res Int* 286390: 1-9.

Rahman MS (eds) (2007) Handbook of food preservation. 2nd ed Food science and technology. Boca Raton: CRC Press,

Raisagar A, Shukla S (2022) Evaluation of probiotic potential of selected lab cultures. *Asian J Microbiol Biotechnol Environm Sci* 24 (2): 269–274.

Ross RP, Morgan S, Hill C (2002) Preservation and fermentation: past, present and future. *Int J Food Microbiol* 79: 3–16.

Saha US, Misra R, Tiwari D, Prasad KN (2016) A cost-effective anaerobic culture method and its comparison with a standard method. *Ind J Med Res* 144(4): 611-613.

Saud B, Pandey P, Paudel G, Dhungana G, Shrestha V (2020) *In-vitro* antibacterial activity of probiotic against human multidrug resistant pathogens. *Arch Vet Sci Med* 3(1): 31–39.

Sokmen AM, Gulluce H, Askin Akpulat D, Daferera B, Tepe M, Polissiou M, Sokmen F Sahin (2004) The *in vitro* antimicrobial and antioxidant activities of the essential oils and methanol extracts of endemic *Thymus spathulifolius. Food Control* 15: 627– 634.

Sridevi V, Sirisha R, Swapna SN (2015) Screening of probiotic goat milk and cow milk isolates for acid resistance, antagonistic activity and tolerance to antimicrobial activity of spices: Molecular identification of potential probiotic goat milk isolate, G8. *Int J Curr Microbiol Appl Sci* 4(8) : 406–421.

Steele R (2004) Understanding and measuring the shelf-life of food, 1st ed. Woodhead Publishing Limited.

Tianli Y, Jiangbo Z, Yahong Y (2014) Spoilage by alicyclobacillus bacteria in juice and beverage products: Chemical, physical, and combined control methods. *Comprehensive Rev Food Sci Food Safety* 13(5): 771–797.

Zacarías MF, Reinheimer JA, Vinderola G, Kulozik U, Ambros S (2020) Effects of conventional and nonconventional drying on the stability of *Bifidobacterium animalis* subsp. lactis INL1. *Int J Dairy Technol* 73(3) : 625-633. https://doi.org/10.1111/1471-0307.12684