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Antibacterial Activity of *Lactobacillus casei* against Foodborne Pathogens

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ABSTRACT: Use of antibiotic therapy to cure foodborne diseases, imbalance the intestinal microflora which may cause digestive disorders. On the other side, probiotics show both preventive and curative properties and hence, are useful as alternative strategies for foodborne disease prevention and as an alternative to antibiotics. In the present study, the antibacterial potential of probiotic culture against common foodborne pathogens was evaluated in-vitro. Common foodborne bacterial pathogens were isolated from selected food samples and primary identification was done by cultural characterization. For confirmation, molecular characterization was done and foodborne isolates were identified as Escherichia coli, *Staphylococcus aureus, Shigella dysenteriae* and *Salmonella typhi*. In the evaluation of antibacterial potential, the selected probiotic culture *Lactobacillus casei* showed high inhibition capacity against all the isolated foodborne pathogens. Thus, there is a scope to use the selected probiotic bacteria against common foodborne pathogens.

Keywords: Agar overlayed method, Foodborne pathogens, Inhibition capacity, *Lactobacillus casei*, Probiotic.

INTRODUCTION

Nowadays foodborne diseases are increasing globally and cause morbidity and mortality worldwide. It is a serious public health concern. According to World Health Organization (WHO), around 1.8 million people died from diarrheal diseases, largely due to contaminated food and water (Greig and Ravel 2009; Newell *et al.*, 2010). About 600 million cases of food-borne infections with 31 global food-borne hazards caused more than 400,000 deaths (Divyashree *et al.*, 2021). The leading cause of foodborne diseases is pathogenic bacteria. Examples of such bacteria include *Staphylococcus aureus*, *Campylobacter jejuni*, *Clostridium*, *Escherichia coli*, *Brucella*, *Listeria monocytogenes*, *Salmonella* species, *Shigella* species, *Vibrio* species, etc. These pathogens enter the food system through contaminated raw materials, water and water supplies, humans, meat animals, wildlife, and insect vectors (Bhunia and Amalaradjou 2012). To cure foodborne diseases, antibiotic treatment therapy is used but this may cause allergic reactions and develop antibiotic resistance or multi-drug resistance in pathogenic bacteria. To overcome this problem, there is a need forthe application of biological approaches that shows antibacterial activity against foodborne pathogenic bacteria. The antibacterial potential of probiotic cultures against foodborne bacterial pathogens suggests the use of probiotics as an alternative to antibiotics. Consumption of probiotics is also associated with several health benefits such as stimulation of the immune system, managing lactose intolerance, prevention of colon cancer and urogenital symptoms, lowering blood pressure and incidence and duration of diarrhea, reduction of cholesterol and allergic symptoms synthesis, removal of carcinogens, etc. (Parvez *et al.*, 2006). Lactic Acid Bacteria (LAB) are the most common probiotic that has traditionally been used as natural bio preservatives in food and animal feed. In a previous study, probiotic bacteria namely, *Lactobacillus sakei*, *Leuconostoc mesenteroides*, *Leuconostoc lactis*, *Lactobacillus curvatus*, *Pediococcus pentosaceus* and *Lactobacillus sakei* showed antibacterial activity against foodborne pathogens (*Bacillus cereus*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella enterica*). The study also suggested use the of Lactic acid bacteria biofilms to reduce foodborne pathogen contamination in the food industry (Kim *et al.*, 2022).

Among LAB, *Lactobacillus casei* is the most studied species due to their commercial, industrial and applied health potential, food grade and GRAS status (generally recognized as safe) (Gerez *et al.*, 2009; Strom *et al.*, 2005). *Lactobacillus casei* also received higher attention because it is a part of human and animalmicrobiota (Casey *et al.*, 2004) and is also found in a variety of naturally fermented food products (Ao *et al.*, 2012; Owusu-Kwarteng *et al.*, 2015). The antibacterial potential of *Lactobacillus casei* might be due to the competitive exclusion of pathogens and the production of antimicrobial substances such as organic acids, bacteriocin, and hydrogen peroxide (Nur and Aslim 2010). Taking into consideration of following points, *Lactobacillus casei* was selected as a probiotic in the present study. The main objective of the present study was to evaluate the antibacterial activity of probiotic bacteria against isolated foodborne pathogens.

MATERIALS AND METHODOLOGY

Isolation of foodbornepathogenic bacteria. In the present study, foodborne bacterial pathogens were isolated from selected dairy and food samples. A total of 200 samples were collected for this purpose. Isolation was done by serial dilution followed by the pour plate method on selective media (Aneja, 2009). After the suitable incubation period, pure cultures of isolates were maintained and storedfor further studies.

Cultural Identification of isolated pathogenic bacteria. Cultural characterization was done by streaking on selective media and microscopic observations were done by Gram's staining method. To perform Gram staining, a thin smear was prepared on a clear dry slide. After air drying and heat fixing, the smear was flooded with Gram's Crystal Violet for 1 minute. The stain was drained out and again flooded with Gram's Iodine for 1 minute. After this, decolorization was done with Gram's Decolorizer. After washing with tap water, counterstained with 0.5% w/v Safranin was done for 1 minute. After washing, the slide was allowed to air dry and examine under an oil immersion objective (Aneja, 2009).

Molecular Identification of isolated pathogenic bacteria. Molecular characterization of the isolates was done by the Sanger sequencing method.In this method, the Genomic DNA of the isolates was extracted using MagMax total nucleic acid isolation kit. After extraction, quantification of isolated DNA was done using a Quantusfluorometer. The specific regions of 16srRNA were amplified by PCR by using universal primers and purified with agarose gel and a PCR clean-up system. After purification, the DNA concentration of PCR products was estimated by Quantus fluorometer and the integrity was checked on EtBr-stained agarose gel (1%). The cycle sequencing was carried out in a heated lid thermal cycler with a diluted sample up to 10ng/µl. After Post sequencing clean-up, the sequence chromatograms were viewed using Chromas software and then aligned to respective 16s reference sequences using BLAST (Basic Local Alignment Search Tool) software developed by NCBI.

Procurement and Maintenance of *Lactobacillus casei*. For the present study, *Lactobacillus casei*was selected as probiotic culture, as it showed probiotic potential in our earlier study (Raisagar and Shukla 2022). The selected probiotic culture*Lactobacillus casei*was procured fromthe National Collection of Industrial Microorganisms (NCIM), Pune in dried culture form. Reviving of culture was done in MRS (De Man Rogosa Sharpe) agar slants in aerobic condition (incubation temperature 37°C; incubation time 24 hours). For further studies, cultures were stored at 4°C.

Antibacterial activity against Foodborne pathogens. The antibacterial activity of the probiotic culture *Lactobacillus casei* against isolated foodborne pathogens was determined by the agar overlay method (Aween *et al.*, 2012). Firstly, media *i.e.*, De Man Rogosa Sharpe (MRS) agar and Muller-Hinton agar was prepared. In the MRS agar plate, *Lactobacillus casei* was inoculated by the spread plate method (Sanders, 2012) and incubated at 37°C for 24hrs. After the proper growth, *Lactobacillus casei* was transferred to a new MRS agar plate in spot form with the help of a 6mm borer. On the other side, molten MHA media was inoculated with isolated foodborne bacterial pathogens. For each pathogen, a separate preparation was done. Now, this molten Muller-Hinton agar media containing a single indicator strain of foodborne pathogen was overlayed in the new MRS agar plate containing *Lactobacillus casei* culture in spot form. The plates were incubated at 37°C for 24 hours. After incubation, the inhibition zone was recorded.

Interpretation of the results. After recording the inhibition zone, the width of the clear zone (R) was calculated by using the formula suggested by Carasi *et al.* (2014); Pisano *et al.* (2014). The used formula was:

$R = dInhib - dSpot/2$

Here,

$dInhib$ = the diameter of the clear zone around the 'Spot' and

$dSpot$ = the diameter of the spot from the growth of probiotic culture on the MRS agar plate.

The calculated R was used to determine the inhibition capacity or inhibition score. If R < 2 mm, it was considered as the no inhibition capacity; R = 2 to 5 mm means low inhibition capacity, and R > 6 mm means high inhibition capacity.

RESULT AND DISCUSSION

Isolation of foodborne pathogenic bacteria. A total of 191 bacterial isolates were isolated from selected samples. By morphological and molecular characterization, isolates were identified as 29.84% *Escherichia coli*, 26.7% *Staphylococcus aureus*, 19.90% *Shigella dysenteriae*, and 23.56% *Salmonella typhi* (Fig. 1).

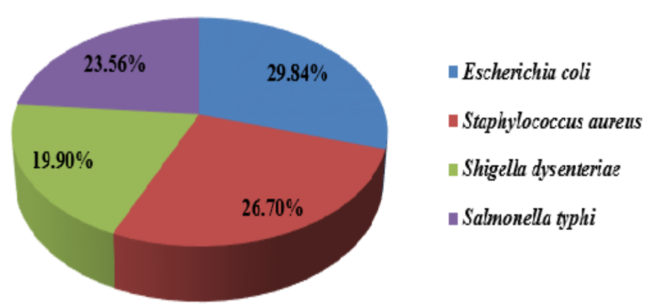
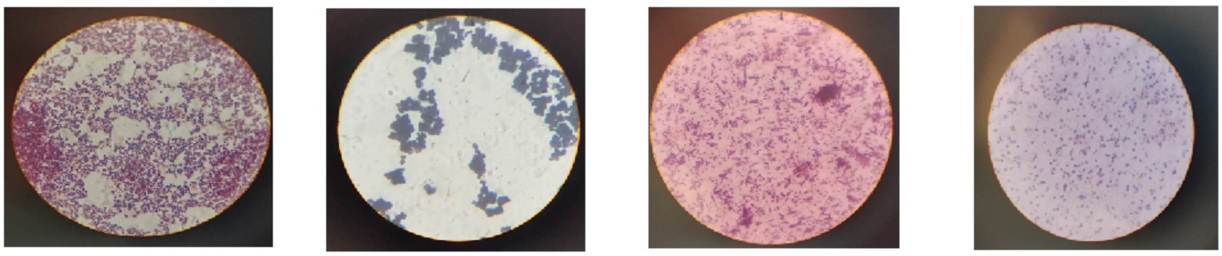


Fig. 1. Isolation of foodborne pathogens.

Cultural Identification of isolated pathogenic bacteria. In cultural characterization, the colony was white, yellow, colorless and grayish-white, for the isolates *Escherichia coli*, *Staphylococcus aureus*, *Shigella dysenteriae* and *Salmonella typhi*, respectively. The colony surface was smooth for all the isolates, except, *Escherichia coli*, which showed a glistening surface. All the isolates showed entire colony margins and circular colony form. Convex elevation was shown by *Staphylococcus aureus* and *Shigella dysenteriae* whereas *Escherichia coli* showed flat elevation and raised elevation was noted with *Salmonella typhi*. Both *Escherichia coli* and *Staphylococcus aureus* showed opaque optical density while transparent and translucent optical density was recorded with *Shigella dysenteriae* and *Salmonella typhi* respectively. In microscopic observation, all the isolates were Gram-negative rods, except, *Staphylococcus aureus* which showed positive for gram's reaction and cocci-shaped cells (Fig. 2).



(a) (b) (c) (d)

Fig. 2. Microscopic Observation of isolated foodborne pathogens (a) *Escherichia coli*, (b) *Staphylococcus aureus*, (c) *Shigella dysenteriae*, (d) *Salmonella typhi*.

Molecular identification of isolated pathogenic bacteria. In molecular characterization, the BLAS result of *Escherichia coli* showed 100% similarities with *Escherichia coli* strain YKUTI708 (Accession no. MF356959.1), query length was 402 and E-value was 0.0. BLAS result of *Salmonella typhi* showed 100% similarities with *Salmonella enterica subsp. enterica serovar typhi* (Accession no. U88545.1), query length was 361 and E-value was 0.0. BLAS result of *Shigella dysenteriae* showed 100% similarities with *Shigella* sp. 09-M2 (Accession no. KC920587.1), query length was 419 and E-value was 0.0.

Antibacterial activity of *Lactobacillus casei*against isolated Foodborne pathogens. The selected probiotic culture *Lactobacillus casei* showed positive antibacterial activity against foodborne pathogens, namely, *Escherichia coli*, *Staphylococcus aureus*, *Shigella dysenteriae* and *Salmonella typhi* in agar overlayed method. The recorded zone of inhibition was 32 mm against *Escherichia coli*, 26 mm against *Staphylococcus aureus*, 22 mm against *Shigella dysenteriae* and 19 mm against *Salmonella typhi*. In the present study, the width of the clear zone (R) was calculated using the formula. The calculated R for *Lactobacillus casei* was 13 mm against *Escherichia coli*, 10 mm against *Staphylococcus aureus*, 8 mm against *Shigella dysenteriae* and 6.5 mm against *Salmonella typhi*. The calculated R values indicated that high inhibition capacity was shown by probiotic culture *Lactobacillus casei* against all isolated foodborne pathogens. (Table 1 and Fig. 3).

DISCUSSIONS

In the present study, the selected probiotic culture *Lactobacillus casei* showed the highest zone of inhibition against *Escherichia coli* while the lowest zone of inhibition was recorded against *Salmonella typhi*. Antibacterial activity of probiotic cultures against foodborne pathogens was also reported previously in several studies (Belicová *et al.*, 2013; Karami *et al.*, 2017; OBdak *et al*., 2017; Moghadam *et al.*, 2018). Similar to the present study, the antibacterial activity of probiotics was also studied by Forhad *et al.* (2015) where *Lactobacillus casei* recorded 14 mm, 18 mm and 12 mm zone of inhibition against pathogenic bacteria *Escherichia coli*, *Salmonella* spp., and *Shigella* species, respectively. Along with *Lactobacillus casei*, the antibacterial activity of *Lactobacillus fermentum*, *Lactobacillus acidophilus* and *Bifidobacterium*species was also studied by Forhad *et al.* (2015). Similarly, the antibacterial activity of *Lactobacillus casei* against *Escherichia coli*, *Staphylococcus aureus* and *Shigella* species was also conducted by Shokryazdan *et al.* (2014) where the recorded zone of inhibition was 13-14 mm, 19-20 mm and 16 mm, respectively. In another study done by Cunha *et al.* (2013), the antibacterial activity of *Lactobacillus casei* against *Escherichia coli* and *Staphylococcus aureus* was well documented. Similar to the present study, Pathak and Dutta, 2016 also selected foodborne pathogens *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Shigella dysenteriae* in their study and the recorded zone of inhibition was 5.5-13.5mm, 0-12 mm, 0-17 mm and 0-16 mm, respectively by using probiotic culture *Lactobacillus acidophilus*. By utilizing theantibacterial potential of probiotic bacteria, Kim *et al.* (2022) developed an antagonistic LAB biofilm that inhibited more than six logs of foodborne pathogenic bacteria. The antibacterial activity of selected probiotic culture could be explained by the production of antimicrobial substances/metabolites, such as organic acids (lactic acid and acetic acid), hydrogen peroxide, diacetyl, acetaldehyde, acetoin, bacteriocins, carbon dioxide, ethanol, reuterin and reutericyclin. The inhibition of pathogens might be also because of the mechanism of competitive exclusion. Competition between probiotic strains and foodborne pathogens for nutrients and attachment sites would prevent the colonization of these pathogens in the gastrointestinal tract.

Table 1: Antibacterial activity of *Lactobacillus casei* against foodborne pathogens.

	Isolated foodborne pathogens			
	Escherichia coli	***Staphylococcus aureus***	***Shigella dysenteriae***	***Salmonella typhi***
Zone of Inhibition (in mm)	32	26	22	19
Width of clear Zone (R) (in mm)	13	10	08	6.5
Inhibition capacity	High	High	High	High

R < 2 mm= no inhibition capacity; R = 2 to 5 mm =low inhibition capacity; R > 6 =high inhibition capacity

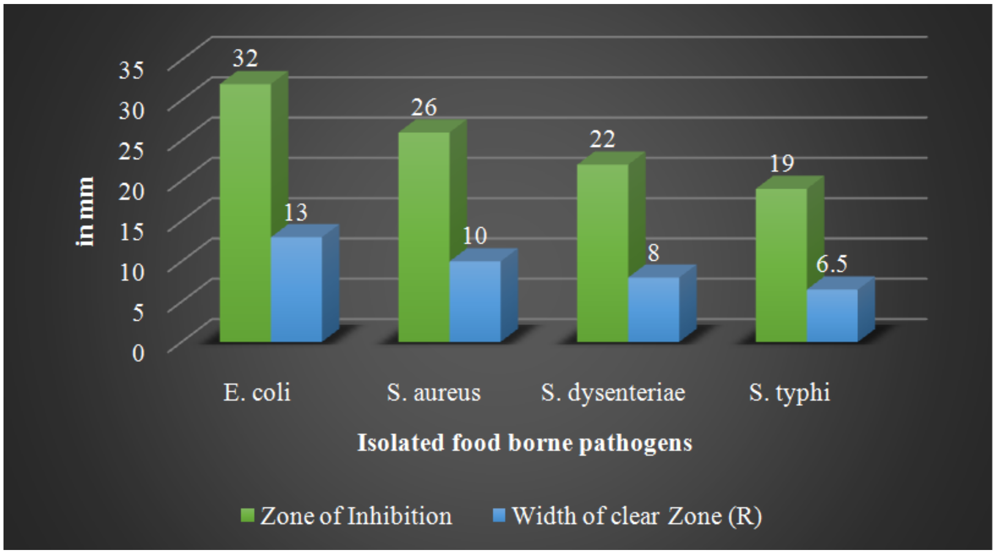


Fig. 3. Antibacterial activity of *Lactobacillus casei* against foodborne pathogens.

CONCLUSIONS

From the present study, it is concluded that the selected probiotic culture *Lactobacillus casei* possesses antibacterial activity against common foodborne pathogens with high inhibition capacity. Therefore, it could be used against foodborne diseases although there is a need of in vivo trials to assess the health benefits provided to the host. There is also a need of conducting further studies on either the same probiotic or on other probiotics against different foodborne pathogens, which proves the use of probiotics against a broad range of pathogens.

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Conflict of interest. None.

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