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Exploration of Indian Traditional recipe "Tarvaani" from the drained rice gruel for nutritional and probiotic potential

Anwesha Chowdhury [a], Monika Choudhary [a], Vidushi Sharma [a], Anil Kant [a], Jitendraa Vashistt [a], Vijay Kumar Garlapati [a,**], Jesus Simal-Gandara [b,*]

[a] Dept.of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Waknaghat, HP, 173234, India
[b] Universidade de Vigo, Nutrition and Bromatology Group, Analytical Chemistry and Food Science Department, Faculty of Science, E-32004, Ourense, Spain

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ABSTRACT

Study background: Traditional fermented foods are the source of probiotic bacteria which can be envisaged as formulation ingredients in various food and beverages.
Scope and approach: The present research aimed to explore one of the Indian traditional recipes, "Tarvaani," for nutritional and probiotic potential as a part of its healthy perspectives.
Main findings and conclusion: The traditional recipe was found to contain the lactic acid bacteria and isolated. The isolated product showed prominent curdling activity after 48 h, and bacterial growth was seen in all the pH (pH 3–7) tested. The antibiotic susceptibility test found that streptomycin, vancomycin, and kanamycin were resistant to the bacterial culture, and imipenem, gentamycin, rifampicin, and tetracycline were found to be resistant susceptible to the bacterial culture according to zone diameter interpretative criteria given in ICMR SOP 2015. A clear halo zone around the tested colony was obtained in the amylase test, which indicated that starch was degraded and α-amylase was produced. The culture also showed prominent antimicrobial action and inhibited the growth of the pathogenic strains tested by a well-diffusion assay. Moreover, the isolates only showed no haemolysis activity after incubation at 30 °C for 24 h under anaerobic conditions. Overall, the present research findings showcase the nutritional and probiotic potential of Lactobacillus sp. of "Tarvaani" as a viable option as a formulation ingredient in traditional-based functional foods.

1. Introduction

Traditional fermented foods have a rich source of probiotic microorganisms and have an enormous range of benefits, such as increased resistance to malignancy and immune system modulation (Ilango and Antony, 2021). The consumption of fermented foods influences the quality, nutritional availability, and safety of the final product. So, to improve the quality of fermented food, isolating wild-type strains from traditional fermented products can be used as starter cultures in food fermentation (Sharma et al., 2021; Owusu-Kwarteng et al., 2015). As a part of this, several conventional Indian foods which were part of the various healthier diets have been reported, and isolated bacterial strains were explored for the probiotic potential for subsequent proposed probiotic formulations (Akman et al., 2021; Gupta et al., 2021; Mohammed and Çon, 2021; Saidumohamed and Bhat, 2021; Monika et al., 2016).

The traditional foods with probiotic potential were considered functional foods. Probiotics are live microorganisms that, when delivered in adequate amounts, provide a health benefit to the host. The functional/traditional foods contain a colossal number of probiotic strains (Lactobacillus sp. /Bifidobacterium sp.) that help in imparting texture, taste, and longer shelf life to the confined food products (Sagdic et al., 2014), along with the health benefits of detoxification of toxic compounds and degradation of mycotoxins. The probiotics are considered Generally Recognized as Safe (GRAS) food additives and help control the growth of pathogens and spoilage microbes in feed and food (Namasivayam et al., 2014).

Probiotic strains must be screened for necessary functional properties such as the production of antimicrobial compounds, resistance to bile salts and gastric acidity, adherence to gut tissue's ability to modulate immune responses, antibiotic resistance, and producing biogenic amines *in vitro* tests (Nath et al., 2021; Fugaban et al., 2021). Hemolytic activity and antibiotic resistance should also be absent where animal

* Corresponding author.
** Corresponding author.
E-mail addresses: shanepati@gmail.com (V.K. Garlapati), jsimal@uvigo.es (J. Simal-Gandara).

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models must prove safety (Belicová et al., 2013). Probiotic helps modify or alter the composition of micro floras in the gut by introducing beneficial microorganisms. The gut is an obvious target for the growth of these functional foods because it acts as an attachment between the diet and all functions of the body. The potential effectiveness of probiotics depends on the survival and health of bacteria in probiotic products transit through the acidic environment "stomach" to the small intestine to exert the health benefits. The transit through the gut helps in inducing the most losses of viable microorganisms. Gastric juice is one of the most critical barriers to pathogens (Akritidou et al., 2022; Cook et al., 2012; Sahadeva et al., 2011).

Fermented food has provided health benefits in various forms as prepared and consumed in several parts of the world and across distinct cultures. In the present study, we have taken one of the traditional recipes, namely "Tarvaani," most famous in the coastal districts of Andhra Pradesh, India. Tarvaani has been used from ancient times as a remedial measure for diarrhea and digestive problems and as an acidulant/curdling agent. The healing powers of "Tarvaani" are less explored and not well known to the scientific world (https://www.betterbutter.in/recipe/25970/tharvani-charu-fermented-gruel-rasam/). As the traditional-based functional foods market is booming due to pandemic issues, the present study has been targeted to isolate probiotic strain from the traditional recipe "Tarvaani" and explore the nutritional and probiotic potential for subsequent research towards traditional-based functional foods.

2. Materials and methods

2.1. Materials and chemicals

Rice grains and Indian palm leaves were procured from the local market, Waknaghat, HP, India. The media constituents were procured from Himedia, Bombay, India. All reagents and chemicals used were analytical/reagent grade and obtained from Merck, India.

2.2. Preparation of the traditional recipe "Tarvaani" and strain isolation

Rice grains (250 g) were taken in a bowl containing 500 ml of drinking water and soaked for 60 min. After 60 min, the rice was cooked and drained the rice gruel. The drained rice gruel was collected in a container and added 3/4th of the water of the drained rice gruel amount when the drained rice gruel was in a lukewarm condition. The Indian palm date leaves (4–5) were added to the lukewarm drained rice gruel and water mixture. The contents were kept for fermentation for 3–4 days at 32 °C. The microbial growth of the fermented broth was checked periodically for microbial growth on the Petri plates as a testing sample. If no microbial broth was observed, take the tiny amount of the fermented gruel mixture and repeat the fermentation process for 3–4 days with the addition of the fresh 3/4th water till attaining microbial growth, confirming through Petri plate screening. Once the microbial growth was found, the strain was subcultured on MRS agar for future strain identification and nutritional and probiotic abilities.

2.3. Strain identification by gram staining

The bacterial culture was taken on a slide and heat-fixed. A primary stain, i.e., crystal violet stain, was applied on the heat-fixed smear of bacterial culture. Crystal violet stains all cells blue or purple. The slide was gently washed in a gentle stream of tap water for a few seconds, and Grams iodine was applied further and left for 1 min. Then the slide was gently washed with tap water, and ethanol was then used to clean the slide for 10 s till the slide ran clear. Counter-stain safranin was then applied and left for 30 s. The slide was then washed for a few seconds under tap water. The results of the staining were observed under a light microscope.

2.4. Determination of nutritional and probiotic potential

2.4.1. Determination of curdling activity

The curdling potential of the sample was checked at two different volumes to see which volume curdling activity was better. In flask 1, a 15 ml sample was added to 60 ml lukewarm milk against a control sample (flask 2, 15 ml buttermilk was added to 60 ml lukewarm milk). In flask 3, a 30 ml sample was added to 45 ml of lukewarm milk against a control sample (flask 4, 30 ml buttermilk was added to 45 ml of lukewarm milk). The curdling activity was checked after 48 h. In all the cases, without a sample, the respective flask contents served as controls (Falfán-Cortés et al., 2022).

2.4.2. Resistance to low pH

The resistance of the strains to low pH was examined. The bacterial culture was first harvested overnight in MRS broth. The bacterial cells from the overnight culture were harvested at 10,000 rpm for 5 min at 4 °C. The cells were then washed with PBS buffer at pH 7.2 twice by centrifugation. The cells were resuspended in PBS buffer, and the pH was adjusted to pH 3.0, pH 4.0, pH 6.0, and 7.0. It was then enumerated on MRS agar. The plates were then incubated at 37 °C for 4 h (Tilwani et al., 2022).

2.4.3. Antibiotic susceptibility

The antibiotic susceptibility was performed using antibiotic discs of Imipenem, Gentamycin, Rifampicin, Streptomycin, Vancomycin, Kanamycin, and Tetracycline. First, inoculation was made with the broth culture diluted to match the 0.5 McFarland Turbidity Standard. The media used for this experiment was Mueller-Hinton Agar. Under aseptic techniques, using a sterile swab, the broth culture of the specific organism was taken. Then, the Mueller Hinton agar plate was streaked using the swab to obtain consistent growth. The plate was rotated five times and then streaked in that direction. Then the antibiotics were dispensed into the plate using flame-sterilized forceps. Plates were incubated overnight at 37 °C (Trindade et al., 2022).

2.4.4. Amylase activity

The bacterial culture was harvested overnight. It was then point inoculated on modified MRS agar without glucose. Starch was used instead of glucose. Inoculated plates were then incubated anaerobically for 48 h at 37 °C. The culture plates were then covered by spraying Lugol's Iodine to detect starch hydrolysis. The production of clear halo zones around the colony indicated starch hydrolysis, i.e., alpha-hydrolysis (Mohd-Zubri et al., 2022).

2.4.5. Antimicrobial activity

Antimicrobial activity was determined against two strains (ATCC 19606 and ATCC 25922) -*Escherichia coli* strain (ATCC 25922) and *Acinetobacter baumannii* strain (ATCC 19606). Fresh overnight bacterial culture was taken. The culture was harvested by centrifugation at 10,000 rpm for 15 min at 4 °C. The bacterial strain's cell-free supernatants (CFC) were tested using a well-diffusion assay for antimicrobial activity. The initial inoculum was incorporated into the MHA, and CFC was also transferred into holes drilled into the agar. The plates were incubated at 37 °C, and the growth-free zone was determined. 20 μl of the culture and supernatant were transferred onto paper, and 100 μl of the culture and the supernatant were transferred onto the wells (Ołdak et al., 2017).

2.4.6. Haemolytic activity

The overnight grown culture was used to perform the haemolytic activity. Blood agar was prepared, and 2 ml of human blood was mixed with the blood agar. The overnight culture was streaked into the blood agar plates. The plates were incubated at 30 °C for 24 h. The reaction can be observed by partial hydrolysis of RBC and α-haemolysis or greening zone, β-haemolysis or clear zone, and ϒ-haemolysis or no reaction

(Pisano et al., 2014).

3. Results and discussion

3.1. Isolation of and characterization of Lactobacillus sp.

The bacterial cultures were isolated from fermented food grains. The Lactobacillus sp. culture was obtained after enrichment in de Man Rogosa Sharpe (MRS) broth (De Man et al., 1960). Further, individual culture colonies were identified on MRS agar and selected for gram staining. The gram staining showed that the bacteria are rod-shaped gram-positive as they are violet in color because of the thick peptidoglycan cell wall (Fig. 1). Hence, the bacteria are identified as Lactobacillus sp. Identification of Lactobacillus sp. from Yunnan traditional fermented yogurt (Jiang et al., 2022), conventional kombucha (Pei et al., 2020) and blown salami packages (Schuster et al., 2019) also executed through the gram staining approach.

3.2. Curdling activity

Lactic acid bacteria in the curd are believed to have probiotic potential, which converts lactose into lactic acid (Balamurugan et al., 2014). Lactobacillus sp. has extensively been used to prepare dairy products such as cheese and yogurt. In the present study, the isolated Lactobacillus sp. showed less prominent curdling activity when added in 1:4 (culture: milk) ratios even after 48 h compared to the control containing the exact proportions of buttermilk and milk. The main curdling activity of the culture, similar to that of control, was observed after 48 h of incubation when a 2:3 proportion of the culture: milk was added for the same final volume. The addition of culture in 2:3 proportions was best for curdling activity after 48 h. The curdling activity was also reported with Lactobacillus sp. isolated from Nigerian unripened soft cheese (Olajugbagbe et al., 2020) and Tenate cheese (Falfán-Cortés et al., 2022).

3.3. Low pH tolerance

The isolated strain was tested for resistance to low pH (pH 3.0–6.0) in PBS at 37 °C for 4 h. The isolated bacteria were viable in all tested low pH. A probiotic bacteria should be capable of surviving under low physiological pH. The low pH of the stomach ranges between pH 1.5 during fasting and pH 4.5 after a meal, which is a tremendous challenge for the survival of these probiotic bacteria before reaching the target site. Survival under very low pH guarantees the isolation of the acid-tolerant strains. The Lactobacillus strains maintained their viability up to pH 3.0 when exposed to various low pH. Our results agree with similar studies that reported that Lactobacillus isolates were viable at a low pH range from pH 2.5 to 4.0 (Owusu-Kwarteng et al., 2015; Argyri et al., 2013; Maragkoudakis et al., 2006). Digested foods inside the stomach contain a high level of fats with specific proteins and are also helpful for the survival of bacteria under low pH of gastric acid. This increases the survival of gastric transit (Ranadheera et al., 2010). The survival of probiotics inside the stomach could be enhanced with selected fermented food (called prebiotics), maintaining the gut microbiota's health (Majeed et al., 2019). The fermented foods often ensure probiotic bacteria are released in the intestine via entrapping the gastrointestinal matrices (Fernández et al., 2015). The low pH tolerance is one of the parameters towards Lactobacillus sp.'s probiotic potential, isolated from Lebanese Baladi goat milk (Saliba et al., 2021) and watery kimchi (Lim et al., 2020).

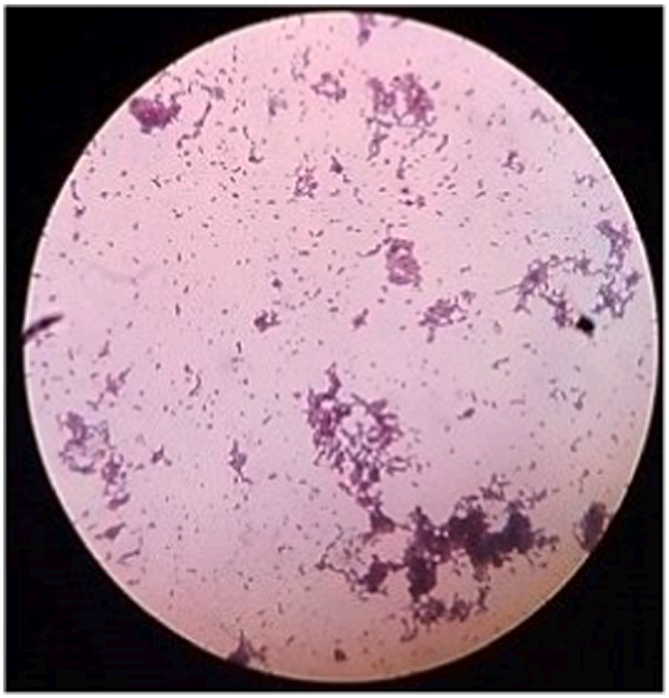

Fig. 1. Gram staining results showing gram positive, rod shaped bacteria.

3.4. Amylase activity

The strains of Lactobacillus sp. isolated from fermented foods were reported as amylase producers (Sanni et al., 2002). After spraying Lugol's iodine over the sample, starch did not stain into a blue-black color, degrading starch. We obtained a clear halo zone around the tested colony, indicating that starch was degraded and α-amylase was produced (Fig. 2). The amylase activity as a part of probiotic potential evaluation also reported with the Lactobacillus sp. towards probiotic enhancement with Gac fruit (Marnpae et al., 2022) and isolated from Murrah buffalo calves (Singh et al., 2021).

3.5. Haemolytic activity of strain

The isolated strain neither showed α-haemolysis (green zone) nor β-haemolysis (clear zone) when grown in blood agar. It only showed γ-haemolysis (i.e., no haemolysis) after incubation at 30 °C for 48 h, as reported by previous studies (Pisano et al., 2014; Maragkoudakis et al., 2006). The absence of haemolytic activity is a crucial safety prerequisite before selecting a probiotic strain (Fig. 3). The absence of haemolytic activity is one of the important consideration of probiotic efficiency of Lactobacillus sp. reported with the isolation studies from fermented milled corn-soybean waste-meal (Nwachukwu et al., 2019) and Haria beer (Das et al., 2022).

3.6. Antibiotic susceptibility test

The antibiotic susceptibility patterns of seven tested antibiotics

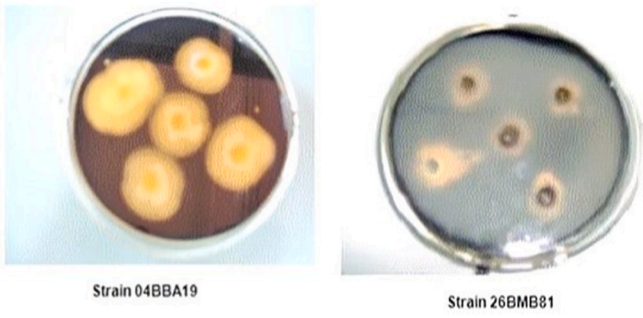

Fig. 2. Amylase test result.

Fig. 3. Haemolytic activity of isolated strain showing grey colonies on blood agar.

against the Lactobacillus sp. were interpreted using CLSI guidelines, and the results are presented in Table 1. The diameter of the inhibition zone of the Lactobacillus sp. showed susceptibility for gentamycin, rifampicin, tetracycline, and imipenem (Fig. 4). The absence of a clear zone of inhibition against streptomycin, vancomycin, and kanamycin indicates the resistance of Lactobacillus sp. against these antibiotics. The resistance profiles of Lactobacillus sp. were similar to the previous reports (Li et al., 2020; Adimpong et al., 2012). Antibiotic resistance of probiotic microorganisms has many viewpoints so far. For example, antibiotic resistance may be desirable to probiotic bacteria to cause diarrhea, which may be advantageous in inducing pathogenesis (Temmerman et al., 2003). On the other hand, Lactobacillus sp. may help reinstate and sustain the normal gut microbiota during antibiotic therapy. To accomplish this, Lactobacillus sp. must be able to persist in the presence of co-administered antibiotics. Hence, antibiotic resistance is a vital criterion for screening and selecting the probiotic, provided they do not risk spreading antibiotic resistance to other bacteria (Jose et al., 2015). The antibiotic resistance is a pre-requisite parameter towards a probable probiotic ability and reported with the *Lactobacillus crispatus* YIT 12319 (Terai et al., 2020) and Lactobacillus sp. (Das et al., 2020).

3.7. Antimicrobial activity

The antimicrobial potential of each raw fermented food and grown bacterial cultures were determined against *A. baumannii* ATCC 19606 and *E. coli* ATCC 25922. The cell-free supernatant and whole-cell culture of each type were tested, where 20 µl of the grown cell culture and cell-free supernatant showed prominent antimicrobial action against both tested isolates compared to raw fermented food. Simultaneously, when increased concentration (i.e., 100 µl of each type)was tested, the raw fermented food also showed antimicrobial activity against tested strains, as shown in Fig. 5. These results indicate that the Lactobacillus strain in raw fermented food has potent antimicrobial activity against A. baumannii and *E. coli*. Similar antimicrobial activities of Lactobacillus strains have been reported by various studies (Dasari et al., 2014; Owusu-Kwarteng et al., 2015; Ołdak et al., 2017). The antimicrobial activity as a part of their probable probiotic activity was also reported with the Lactobacillus spp. (Azizian et al., 2021) and Lactobacillus acidophilus LaCH-5 (Saarela et al., 2007).

Table 1
Antibiotic susceptibility profiles of *Lactobacillu* isolate.

Antibiotics	Zone of inhibition (mm)	Susceptible (S)/Resistant (R)
Gentamycin	20 ± 1.02	S
Rifampicin	25 ± 0.45	S
Tetracycline	18 ± 0.95	S
Imipenem	42 ± 0.75	S
Streptomycin	0	R
Vancomycin	0	R
Kanamycin	0	R

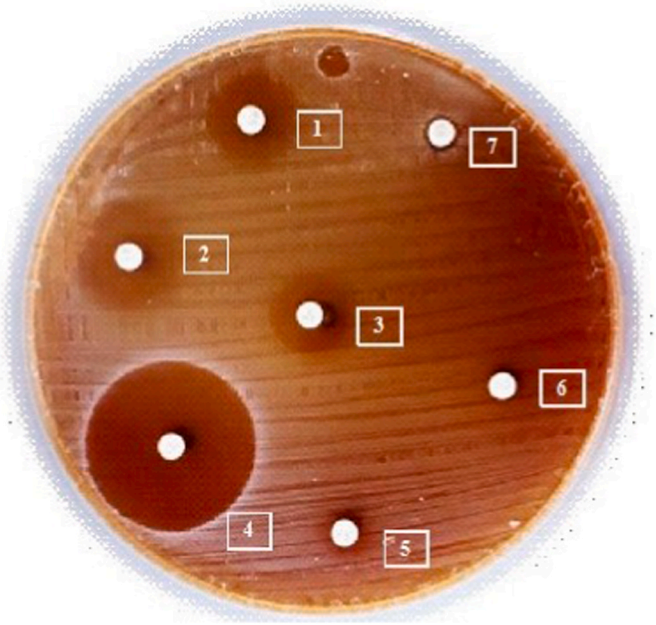

Fig. 4. Antibiotic susceptibility patterns of tested antibiotics against Lactobacillus sp.. Clear inhibition zone showed the susceptibility of Lactobacillus sp. against (1) gentamycin, (2)rifampicin, (3) tetracycline, (4) imipenem. No clear zone of inhibition was observed against (5) streptomycin, (6) vancomycin, and (7) kanamycin indicated the resistance of Lactobacillus sp..

4. Conclusion

The present study put forth the magical health healing capabilities of the Indian traditional recipe "Tarvaani" by isolating the *Lactobacillus* strain. The isolated *lactobacillus* strain has been further evaluated for nutritional and probiotic potential through low pH resistance, curdling. The isolated *Lactobacillus* sp. strain was also evaluated for antimicrobial and executed the antibiotic susceptibility and didn't exhibit any haemolytic activity. Thus, the culture can be used as a probiotic product as it can be easily prepared at home and is a cost-effective option as a probiotic food. Therefore, the isolated *Lactobacillus* sp. is a viable option as a formulation ingredient in different probiotic beverages and traditional-based functional foods. Overall, the study introduces one of the Indian Traditional recipes, "Tarvaani," to health researchers for its nutritional and probiotic potential with foreseeable formulation ingredients for functional food-based beverages and products in nearby future.

CRediT authorship contribution statement

Anwesha Chowdhury: Investigation, Formal analysis, Validation, Visualization, Writing – original draft. **Monika Choudhary:** Investigation, Formal analysis, Writing – review & editing. **Vidushi Sharma:** Investigation, Formal analysis, Validation, Visualization, Writing – original draft. **Anil Kant:**Methodology, Writing – review & editing. **Jitendraa Vashistt:**Methodology, Writing – review & editing. **Vijay Kumar Garlapati:** Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition. **Jesus Simal-Gandara:** Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition.

CRediT author statement

All authors equally contribute to Conceptualization, Methodology, Formal Analysis, Investigation, Writing, and Visualization, under

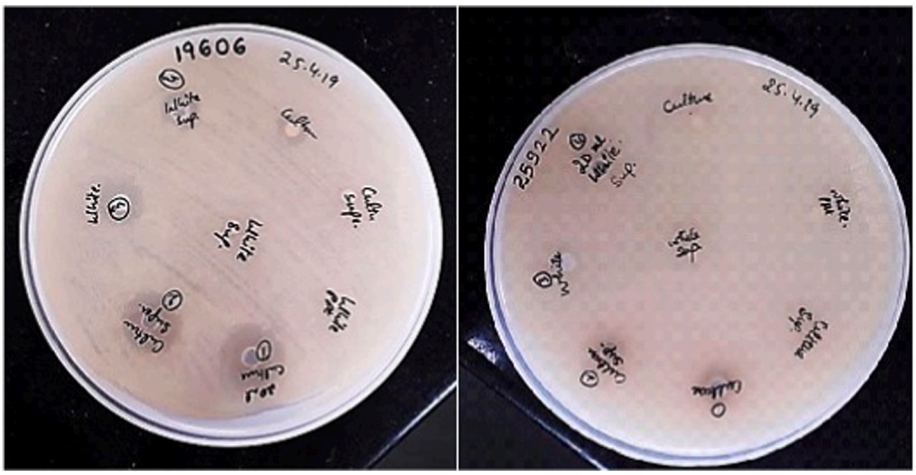

Fig. 5. Antimicrobial activity at different concentrations of cell free supernatant and whole cell culture of raw fermented food and grown bacterial culture againts *A. baumannii* ATCC 19606 and *E. coli* ATCC 25922.

Supervision of the corresponding authors.

Implications for gastronomy

Fermented food has provided health benefits in various forms as prepared and consumed in several parts of the world and across distinct cultures. In the present study, we have taken one of the traditional recipes, namely "Tarvaani," most famous in the coastal districts of Andhra Pradesh, India. The healing powers of "Tarvaani" are less explored and not well known to the scientific world (https://www.betterbutter.in/recipe/25970/tharvani-charu-fermented-gruel-rasam/). As the traditional-based functional foods market is booming due to pandemic issues, the present study has been targeted to isolate probiotic strain from the traditional recipe "Tarvaani".

Declaration of competing interest

The authors declare that they don't have any competing interests in executing the present research.

Data availability

Data will be made available on request.

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