RESEARCH   **Open Access**

Biochemical and microbial meta-profiling reveal the anti-cancer efficacy of Eu—an endemic traditional brew of the Toto tribe from India

Soumita Bhattacharjee[1,4], Indrani Sarkar[1], Mahima Misti Sarkar[2], Swarnendu Roy[2], Chandra Ghosh[3] and Arnab Sen[1,4*]

Abstract

Background Eu is a lesser-known and traditional millet-based fermented brew of the Toto tribe from West Bengal, India. The starter culture used in this brew consists of diverse microflora. Identifying those microbes will not only help us gain a deeper understanding of the brew, but also quantify the impacts of this brew on the human system after consumption. Due to the progress in Illumina sequencing technology, the meta-profiling of 16S rRNA has emerged as a valuable resource for exploring the microbiome of any sample. We have utilised this tool (V3–V4 region) to study the microbiome population of Eu.

Results The microbial diversity present in the brew was mainly constituted of probiotic and fermenting bacteria. The nutrient profiling of Eu estimated the amount of protein, carbohydrate, free amino acid, and free fatty acid. The nutritional substance present in the brew probably supports the growth of the probiotic microflora. Reverse ecology-based network analysis revealed considerable complementary interaction between humans and the microflora present in the Eu. GC–MS outlining enlisted thirteen potent compounds from Eu, and a comprehensive network pharmacological study on those compounds revealed the anti-cancerous properties of Eu, especially against gastrointestinal malignancy, by reducing the proliferation of malignant cells and forming pro-inflammatory cytokines.

Conclusions The current study focuses on the meta-profiling and nutritional characteristics of Eu, which highlight the beverage's potential as a probiotic health drink. Reverse ecology analysis demonstrated that the probiotic microflora competes strongly against pathogenic bacteria. Additionally, in silico network pharmacological research identified the drink as a potentially health-promoting and anti-cancer beverage.

Keywords Traditional beverage, Meta-profiling, Nutrition, Starter culture, Microflora, Lactic acid bacteria, Reverse ecology, Network pharmacology

*Correspondence:
Arnab Sen
arnab.nbu@gmail.com
Full list of author information is available at the end of the article

Background

The cultural significance of alcoholic beverages traces back to the ancient Indus Valley Civilization. In India, various tribal communities have standardised the art of crafting ethnic brews (Tamang 2020). Often locally consumed, these beverages feature indigenous microbial consortia added during preparation. The microbiota present in these drinks may augment the digestibility of foods, enriching nutritional quality (Dridi et al. 2023). The 'Toto' tribe in West Bengal, India, a primitive mongoloid Indo-Bhutanese isolated group, relies heavily on woodland produce for their livelihood. One of their unique traditional drinks is Eu (/ju:/), a millet-based brew with low alcohol content and high antioxidant value (Bhattacharjee et al. 2021). Eu is characteristically prepared from fermented millet seeds with a spherical rice ball ('Moaa/Moi/moe'), incorporating various locally grown herbs (*Capsicum annuum* L, *Plumbago zeylanica* L., *Drymaria cordata* (L.) Willd. ex Schult., *Athyrium nigripes* (Blume) T. Moore*, Pterospermum acerifolium* (L.) Willdenow).). These rice balls serve as amylolytic starter cultures, harbouring diverse microbial groups that influence the biochemical properties of the beverage.

Nevertheless, traditional culture-dependent analyses of the microbial world have limitations, prompting the espousal of next-generation sequencing technologies like meta-profiling studies. In this context, the microbial population of the amylolytic starter culture, especially through meta-profiling, remain rather uncharted. This approach can offer a novel back-slopping methodology for cultivating native microbiota in the preparation of fermented beverages. Fermented beverages, such as Eu, not only extend the shelf-life of raw ingredients, but also introduce probiotic microflora into the system. These probiotic microbes may enhance the host immune system, contributing to overall health benefits (Das et al. 2017; Patel et al. 2014). Considering the complex biological interactions among the microbes, reverse ecology analysis emerges as a valuable tool for transforming high-throughput genomic data into ecological interactions. However, despite studies on the bioactive constituents of Eu, their effects on human diseases remain largely illusive. Network pharmacology, an in silico analytical approach, could light up the potential effectiveness of Eu-derived compounds, especially alongside gastrointestinal malignancies. Recognising the declining tribal populations in India due to shifts in livelihood and urban relocation, preserving the indigenous knowledge of communities like the 'Toto' becomes crucial. In light of these considerations, our study focuses on the 'Eu' utilising metagenomics and bioinformatics approaches. Our objectives cover determining Eu's nutritional makeup, exploring microbial associations in the starter culture through NGS meta-profiling, investigating microbial interactions with reverse ecology and network analyses, and conducting a network pharmacological study on Eu's potential efficacy against gastrointestinal cancer.

Methods

Sample collection

Freshly prepared ten tablets of amylolytic starter culture (Moaa/moi/moe) were collected from the rural houses in Totopara near Madarihat, at 26° 49′ 48.00″ N latitude and 89° 18′ 36.00″ E longitude in the Alipurduar District of West Bengal on February 2020 (Additional file 1). Three bottles of freshly prepared Eu were also collected in Borosilicate Glass Reagent Bottles (sample volume: 1000 mL). The samples collected in Totopara were brought to the laboratory in ice packs, and the starter and the liquid were kept in the refrigerator at 4 ℃ for further proximate analysis.

Nutritional composition of Eu

The collected liquid sample (Eu) was subjected to analysis of the biochemical composition and the nutritional value using the standard protocol. Previously, we have done a lyophilised methanolic extraction of Eu for GC–MS analysis to obtain the bioactive compounds in the brew (Bhattacharjee et al. 2021).

Moisture content

The moisture content was estimated following the method defined by McCleary (2023). 5 mL of the sample was weighed (FW), then the same sample was oven-dried until completely dried. The dry weight (DW) of the sample was recorded and the following formula calculated the moisture content

$$Moisture content(\%) = \frac{(FW - DW)}{FW} \times 100$$

Ash content

Ash represents the presence of inorganic mineral content in the drink. The ash content has been determined as a routine analysis to evaluate the quality of traditional beverages. The samples with high ash content could indicate high mineral content (Bayoï et al. 2021). A 5 mL sample was calcinated at 500 ℃ for one hour to obtain the ash content. The ash content was expressed as a mg/mL sample.

Estimation of free fatty acid and free amino acid

Free fatty acid was estimated using Cox and Pearson's standard method (1962). The estimation of free amino acids was done following the method of Moore and Stein (1948). The absorbance of the purple-coloured reaction

mixture was finally taken at 570 nm against the blank after 15 min.

Estimation of total and reducing sugar

Total sugar content was determined using Anthrone reagent following the standard method of Plummer (1978). The absorbance of the developed blue colour was measured at 515 nm.

Estimation of protein

Soluble protein was determined using the method of Bradford (1976). The absorbance of the colour change was measured at 595 nm, and protein was calculated using a standard curve of Bovine serum albumin (BSA).

Meta-profiling
DNA extraction of the starter culture

The amylolytic starter culture or dry rice tablets (Moaa) were ground in a motor pestle. 3 g of powdered sample was dissolved and homogenised in 20 mL of 0.84% NaOH solution. The samples were then filtered using filter paper (pore size: 11 μm and diameter: 44 mm). The final filtrate was kept for DNA extraction using a kit (QIAGEN, Germany). DNA of the sample was kept at − 20 ℃ temperature until further use. In addition, V3–V4 region-based 16S Metagenomic amplicon sequencing was also done and analysed.

PCR amplification 16SrRNA (V3–V4 based)

Illumina™ Nextseq platform was used for the 16SrRNA. The bacterial 16S rRNA (V3–V4) region was amplified using the primer sequences of the forward strand: 5'AGA GTTTGATGMTGGCTCAG3'(V13F) and 5'TTACCG CGGCMGCSGGCAC3'(V13R) for the reverse strand. The polymerase chain reaction (PCR) was done with the first denaturation step at 95 ℃ (15 s), annealing at 60 ℃ (15 s), elongation at 72° (2 min), and the final extension step for 10 min and hold at 4 ℃ for 25 cycles (Saxena et al. 2021). The extracted DNA (40 ng) and 10 pM (picoMolar) of each primer were used for amplification. The amplicons were purified for each sample with 1X Ampure beads (Xploregen Discoveries) to remove unused primers. The purified PCR product was subjected to Gel check and Nanodrop QC. The Nanodrop readings of 260/280 at an approximate value of 1.8–2 were taken to determine DNA's quality. For preparing the sequencing libraries, eight cycles of PCR were performed using Illumina barcoded adapters. The Ampure beads Libraries were purified and quantified using a Qubit dsDNA High Sensitivity assay kit. IlluminaMiseq with a 2×300PE v3 kit was used for Sequencing.

Processing of metagenomics data

FASTQC and MULTIQC were used to analyse raw data QC. Data lost after QC = 2968 bp. TRIMGALORE was used for trimming adapters and low-quality reads. Furthermore, the trimmed reads are taken for processing, including merging of paired-end reads, chimera removal, and OUT abundance calculation. KRAKEN workflow used for Estimation correction. This workflow helps for highly accurate investigations at the genus level, SILVA (https://www.arb-silva.de/), and NCBI (https://www.ncbi.nlm.nih.gov/) databases used. A schematic illustration of the complete 16S metagenomics process is visually depicted in Additional file 2: Fig. S1.

16S metagenomic data for Eu has been submitted in NCBI SRA under the BioSample accession number SAMN29515284 with BioProject ID: PRJNA856115.

Metabolic pathway prediction and reverse ecology study

Reverse ecology analysis provides a relatively new method for investigating interactions among microbes within a specific sample. This analytical approach considers competition and complementation, comprehensively assessing microbial interactions (Sarkar et al. 2022, Bhattacharjee el al., 2023).

The metagenomic analysis detected several predominant genera in the Eu. We used the KEGG and KEGG autonomic annotation server (KAAS) to obtain information regarding the metabolic pathways of considered microbes.

This provided us with all the KEGG Ontology IDs (KO ID) for each gene. Those KO IDs were used for reverse ecology analysis. RevEcoR is open-source software designed for researching microbial community ecology. The RevEcoR programme (https://github.com/yiluh eihei/shiny-RevEcoR.) of *R* was used for this calculation. This programme clearly shows the ecological interaction among the considered strains through competition and complementation index. Cytoscape software visualised a network based on the reverse ecology analysis data.

The metagenomic study identifies microbial diversity from phylum to genus level. We have taken a cut-off value of 100 sequence count, and if the value is below 100 for a specific genus, we have not considered it. This was done to keep a controllable quantity of output data and to eliminate the possibility of false positives.

In silico anti-cancer study
Prediction of potential genes associated with gastrointestinal cancer

From the GCMS analysis, 13 bioactive compounds were detected in Eu. The SMILES of each compound were obtained from the online smile server and uploaded to

the Superpred server for target prediction of each compound. Predicted targets with more than a probability score of 90% were taken for further analysis. This list of the unique genes from each compound was uploaded on the DisGeNET server (https://www.disgenet.org/). DisGeNET is the database where we can find the target disease correlation. The obtained results were sorted for cancers related to the digestive system. These results were incorporated within Cytoscape 3.9.1 (https://cytoscape.org) to evaluate biomolecule interactions and network visualisation (Shannon et al. 2003).

Results

Nutritional composition

The sample has low alcohol content. Various bioactive compounds were identified in the GC–MS analysis of Eu. Through GC–MS analysis in Eu, lactic acid synthesis by lactic acid bacteria benefits human health by limiting the

growth of pathogens and working as an immune stimulator (Das et al. 2017). The protein content of the sample is 0.054 mg/mL, whereas the free amino acid content is 0.008 mg/ mL. Total starch content, reduced sugar, and soluble sugar were calculated to estimate total carbohydrates. The starch content is 1.178 mg/mL, total soluble sugar is 0.074 mg/mL, and reducing sugar is 0.015 mg/ mL. The free fatty acid content is 0.109035 mg/mL. Ash value provides the content of inorganic minerals left behind after the water and organic matter have been removed by heating or some other evaporating method. The sample is also rich in mineral content, as indicated by a high ash content (2.76 mg/mL). Other parameters are enlisted in Table 1.

Meta-profiling analysis

Primary data summary, alpha (α) diversity and rarefaction curve

The paired-end reads of the Eu sample gave 52% GC coverage. FasQC report of the sample provided good-quality reads showing successful metagenomic sequencing.

Alpha diversity was determined to find the species diversity in the sample. Alpha (α) diversity of Eu starter culture is 14.08. The species richness in the sample was calculated with the help of a rare fraction curve. This curve generally shows a familiar pattern where, in the first phase, it proliferates for the most common species in the sample, and gradually, it makes a plateau as the rarest species remain to be sampled. For the Eu sample, the rarefaction curve started to form a plateau at the species count 1400 (Fig. 1). The receiver operating curve (ROC) was plotted with the whole microbial diversity of Eu. The

Table 1 Nutritional profiling of Eu (An ethnic brew of Toto tribe)

Sample	Eu
Weight in gm/shape	7.023/round
Moisture content (%)	18.61
Crude protein (mg/mL)	0.054
Total starch (mg/mL)	1.178
Free amino acid	0.008
Total soluble sugar (mg/mL)	0.074
Reducing sugars (mg/mL)	0.015
Free fatty acid (mg/mL)	0.109035
Ash content (mg/mL)	2.76
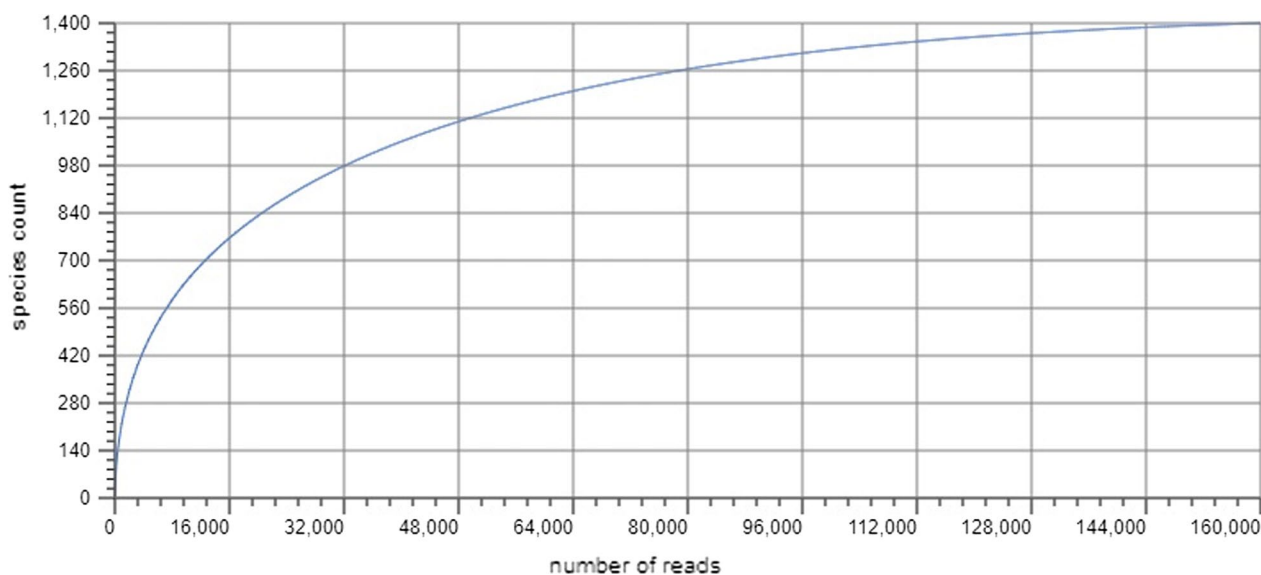
Fig. 1 Rare fraction curve of starter culture

Bhattacharjee *et al. Bulletin of the National Research Centre*   (2024) 48:14

Page 5 of 12

fitted AUC (area under ROC) was 0.905, and the empiric AUC was found to be 0.892 (Fig. 2). The Simpson diversity index was found to be 3.85.

Study on taxonomic level from phylum to genus

The different microbial community present in the starter culture of the Eu is shown in Table 2 and Fig. 3 (a and b).

Ecological interaction among Eu microbial population

The reverse ecology analysis among the microbial population of Eu was performed. Network based on reverse ecology is given in Additional files 3 and 4. The edges of pathogenic bacteria were shown in red, and non-pathogenic bacteria were shown in blue. It was found that the beneficial bacteria of Eu, i.e. LAB and other fermented microbes, are also present in human guts. Those beneficial bacteria were competing with the pathogenic bacteria of the Eu. This exciting finding indicates that the consumption of Eu may enhance the population of good gut microbes in humans. Since gut health is directly related to normal cognitive function, improved gut

Fig. 2 Receiver operating characteristic (ROC) plot analysis of the sample

health after consumption of Eu may also enhance brain functionality (Yang et al. 2020) (Additional file 5). Thus, we may propose that the beneficial microbial population of the Eu will enrich the gut microbes of humans on the one hand and will challenge the pathogenic microbes of the Eu through resource competition on the other.

Network pharmacological study

A total of fifty-six targets were obtained from thirteen compounds of Eu. The result showed Ethyl alpha.-d-glucopyranoside is the most active compound with the highest number of targets. The targets with more than 90% interaction probability were taken into consideration. From DisGeNet, a list of 954 diseases that are associated with the predicted targets was downloaded. Out of these, eighty-two diseases are associated with pancreatic cancer, stomach cancer, colon cancer, and gastrointestinal cancer.

Discussion

All over the world, cereal-based products are the primary sources of dietary nutrients. However, sometimes cereals are deficient in some nutritional components like essential amino acids and micronutrients (Asrani et al 2023). Fermented millet, wheat, and rice beverages are very common and popular among the tribal ethnic groups in India. Eu is one of them and is mainly prepared and consumed throughout the year and in various religious festivals. Preparation of this liquor involves the conversion of cooked millet by its physical, biochemical, and microbiological changes, including inoculation of starter culture and fermentation. Fermentation is the most straightforward and valuable process to increase cereals' nutrient availability and functional qualities (Singh et al. 2015). Modern food processing uses techniques like pasteurisation and thermal heat processing to reduce the growth of microorganisms and raise the shelf-life of the
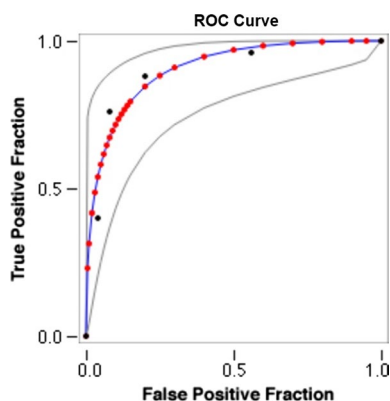
Table 2 Microbial diversity in the starter culture of Eu, from Phylum to Genus level

Phylum	Firmicutes, Proteobacteria, Actinobacteria, Chloroflexi, Cyanobacteria, Deinococcus-Thermus, Chlamydiae, and Planctomycetes
Class	Bacilli, Alphaproteobacteria, Gammaproteobacteria, Clostridia, Actinobacteria, Bacteroidia, Flavobacteria, Negativicutes, Erysipelotrichi, Betaproteobacteria, Planctomycetacia, Deinococci
Order	Lactobacillales, Rhodospirillales, Enterobacteriales, Clostridiales, Bacteroidales, Bacillales, Actinomycetales, Rhizobiales, Bifidobacteriales, Coriobacteriales, Pseudomonadales, Flavobacteriales, Selenomonadales, Erysipelotrichales, Caulobacterales, Burkholderiales, Planctomycetales, Rhodobacterales
Family	Leuconostocaceae, Acetobacteraceae, Enterobacteriaceae, Lactobacillaceae, Clostridiaceae, Ruminococcaceae, Prevotellaceae, Bacillaceae, Bifidobacteriaceae, Rhizobiaceae, Lachnospiraceae, Coriobacteriaceae, Flavobacteriaceae, Bacteroidaceae, Pseudomonadaceae, Micrococcaceae, Eubacteriaceae, Streptomycetaceae, Veillonellaceae, Erysipelotrichaceae, Caulobacteraceae, Bradyrhizobiaceae, Staphylococcaceae, Planctomycetaceae, Rhodobacteraceae, Deinococcaceae
Genus	*Weissella, Gluconobacter, Leuconostoc, Lactococcus, Lactobacillus, Pantoea, Acetobacter, Enterobacter, Clostridium, Prevotella, Bifidobacterium, Bacillus, Serratia, Faecalibacterium, Bacteroides, Agrobacterium, Pseudomonas, Chryseobacterium, Pediococcus, Collinsella, Ruminococcus, Cellulosilyticum, Eubacterium, Arthrobacter, Streptomyces, Robinsoniella, Brevundimonas, Catenibacterium, Staphylococcus, Ethanoligenens, Bosea, Rhodobacter, Deinococcus*
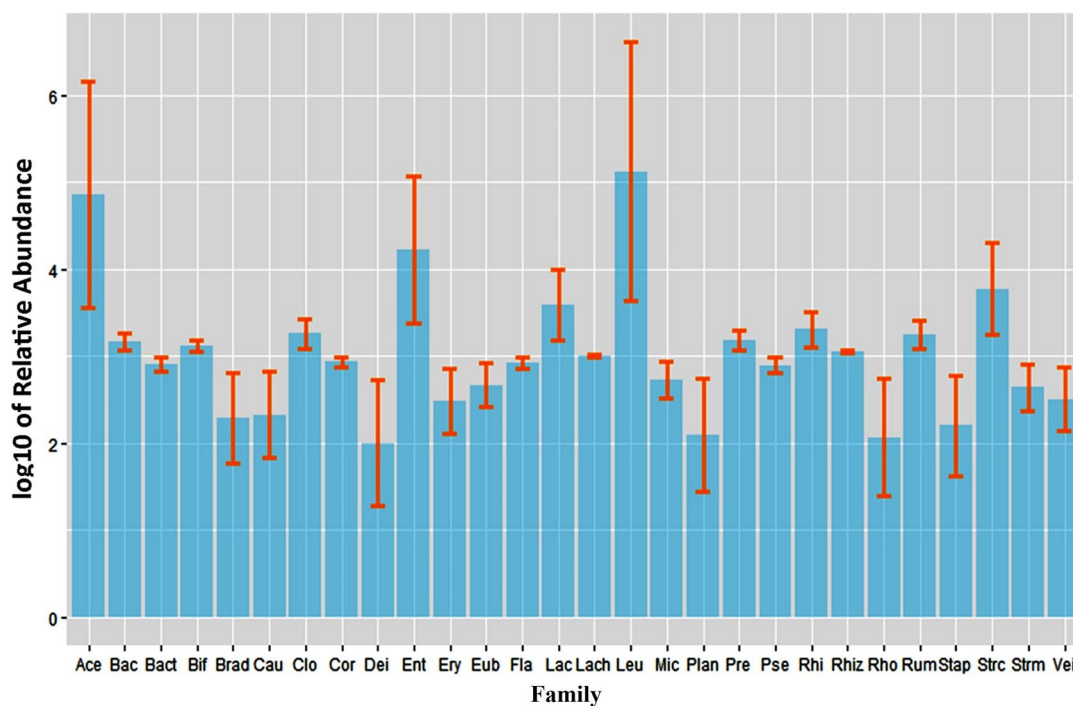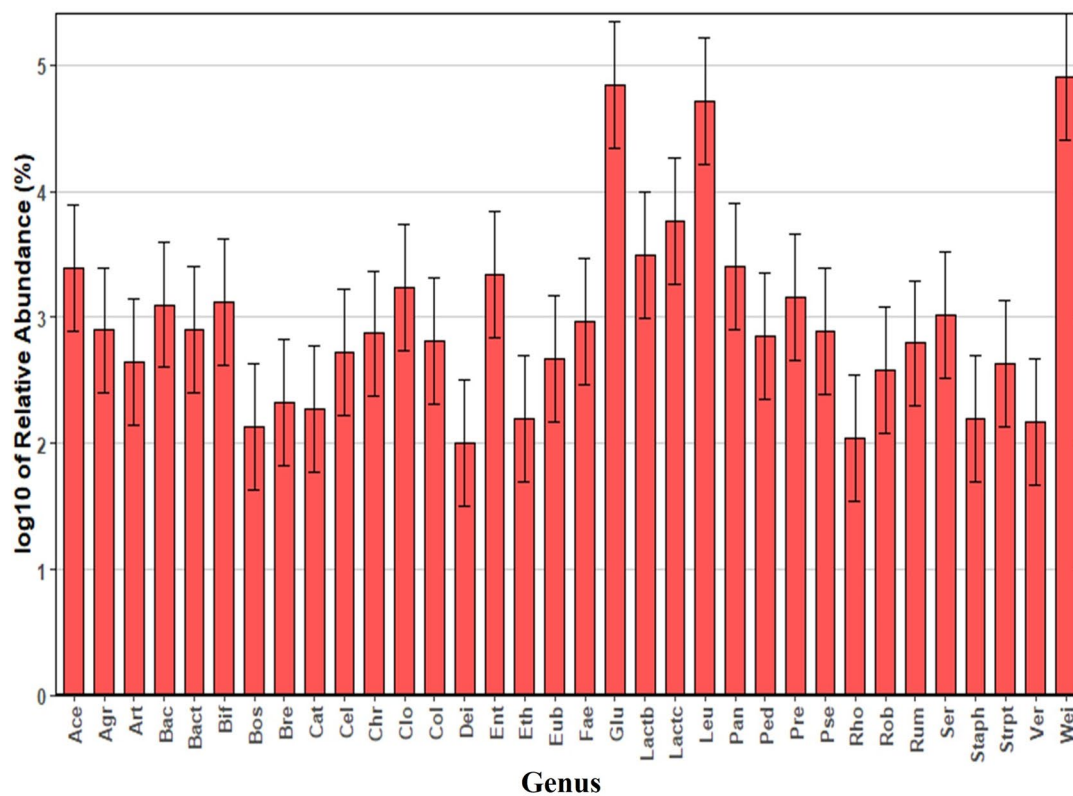
Fig. 3 Microbial abundance of the sample **a** The graph represents the relative abundance of Family in the Log10 scale on the Y-axis, whereas the X-axis represents the different bacterial families. **b** The Y-axis represents the percentage of relative abundance of genera on the Log10 scale and X- the axis represents the different microbial genera present in the sample

food. Different food preservatives are also widely used to reduce food spoilage due to microorganisms. With these modern techniques, in most cases, nutritional value becomes secondary. In contrast, LAB strains within any ethnic fermented food produce organic acids (lactic acid, acetic acid), and hydrogen peroxide, bacteriocins that reduce the growth of some pathogenic microbes within the food matrix, and these probiotic bacteria in Eu, can eliminate constituents like endotoxins and exotoxins (Das et al. 2017), and enrich the brew with malto-oligomers, antioxidants, phenolics, and flavonoids which modulate the sensory quality of the brew. Through GC–MS analysis of Eu, several volatile compounds are detected, mainly including ester, alcohol, and acids. Most of these esters are the by-products of millet fermentation. The nutritional composition reveals that Eu contains many proteins, carbohydrates, free amino acids, and fat. The higher amount of ash in the Eu indicated the presence of more significant amounts of minerals in the beverage. In addition, the food supplements or the prebiotics and non-digestible carbohydrates in Eu promote probiotics and human health by enriching the nutrients and also modulating the gut microflora.

During the fermentation of Eu, a huge range of microflora is involved, but only some of them will determine the taste and quality of the liquor. Fermented millet product or Eu is recognised to be the platform for numerous biochemical and ecological processes where the microbial population plays a vital role in the final conversion of carbohydrates into alcohol and esters. A schematic diagram of the activity of Eu on the human body is shown in Fig. 4. The human body is a lively home for the trillions of bacteria that live symbiotically with the host, especially in the gastrointestinal tract (gut microbiota). The gut microbiota plays a vital role in modulating human overall health. The human gut is occupied by the phyla Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, Fusobacteria, and Verrucomicrobia, and 90% of them are Firmicutes and Bacteroidetes (Stojanov et al. 2020). Firmicutes are the gram-positive bacteria that induce most of the Lactic acid bacteria. Common genera in Firmicutes include *Weissella, Leuconostoc, Lactococcus, Lactobacillus, Enterococcus,* etc.

Lactic acid and acetic acid bacteria prevalent in the Eu

The microbes primarily found in the sample were probiotic LAB and other strains. The major Lactic acid bacterial genera include *Weissella, Leuconostoc, Lactococcus, Lactobacillus,* and *Pediococcus.* Acidic production from this group also favours the growth of some yeast and suppresses the growth of some pathogenic contaminants. Probably, the presence of these LAB strains changes the taste of Eu and provides a hazy appearance to this drink.

LAB strains are commercially used in pharmaceutical industries. *Weissella*, one of the presiding bacteria in Eu, can survive in the gastrointestinal tract and produces antimicrobial exopolysaccharides (Du et al. 2023). Moreover, the ability of *Weissella* to survive in the gastrointestinal tract produces different antimicrobial substances and a variety of compounds that promote the growth of other beneficial gut microbiomes (Teixeira et al. 2021). *Bifidobacterium*, a well-known probiotic strain in Eu, inhibits the growth of other pathogenic contaminants and has active health-promoting properties. *Bifidobacterium* strains inhibit the growth of some pathogenic strains such as *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus,* and *Candida albicans* (Georgievaet al. 2015). *Bifidobacterium* along with *Lactobacillus* can reduce the cholesterol level, inhibit tumours, improve immune responses, and are also reported to prevent diarrhoea (Vinderola et al. 2000). Another LAB strain, *Pediococcusis, is* susceptible to antibiotics and can also increase the expression of Bax protein and decrease the expression of Bcl 2 protein to induce apoptosis (Jafari-Nasab et al. 2021). Acetic acid bacteria (AAB) belonging to Acetobacteraceae, including the genera Gluconobacter, Acetobacter represented in the starter. Acetic acid bacteria can produce acetic acid by the oxidative fermentation of ethanol. Vinegar, an aqueous acetic acid solution, has also been reported for its antibacterial activity and reducing cardiovascular diseases (Gomes et al. 2018). *Bacteroides*, an abundant microflora found in the sample, is known as a next-generation probiotic for its health-promoting activities (Wang et al. 2021).

Environmental contaminants/plant pathogens

Some major environmental contaminants and a few opportunistic human skin pathogens were also detected in the sample: *Pseudomonas, Pantoea, Streptomyces, and Staphylococcus.* These ethnic fermenting methods are standard in rural houses where hygiene is often compromised. This environmental and opportunistic pathogen in the starter culture probably came with the plants' parts or during unhygienic practices.

Reverse ecology analysis

Every species lives in multifarious ecosystems. A microbial community's structural composition reveals diversity and function and the surroundings in which it survives. Conventional ecological methods are limited as they can only be applied on a small scale and for reasonably well-understood biological systems. Recently, a graph-theory-based algorithm known as the reverse ecology approach (an in silico approach) has been established. It can investigate the metabolic links of all the species present in a microbial community and predict their
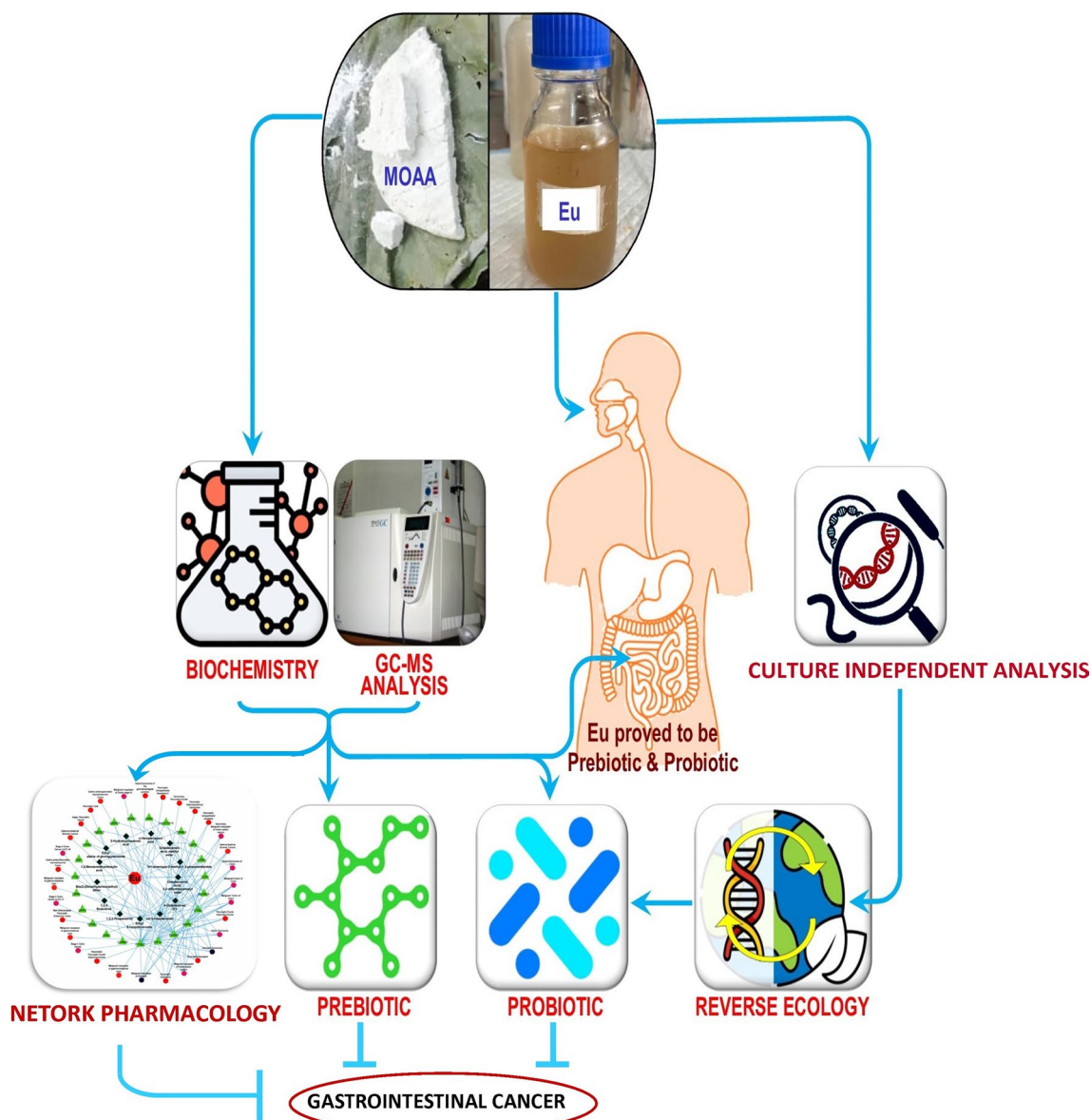
Fig. 4 Schematic representation of the activity of Eu and starter culture (Moaa/moi/moe). Nutritional profiling shows its prebiotic nature. Through reverse ecology analysis, we validated the complementary effect of good bacteria from Eu microbiota on humans as well as the antagonistic effect of pathogens present in Eu on humans

interaction within the environment (Cao et al. 2016). Several research papers demonstrate microbial interactions using this tool, as evidenced by Sarkar et al. (2022) and Bhattacharjee et al. (2023).

Complementation index

The complementation index showed that the beneficial bacterial population was showing complementation with the non-pathogenic microbes. Also, the beneficial bacterial sets complement one another. This shows they have a stable ecological interaction among them, which is better

for any stable ecotype model. Thus here we may say that in Eu, the beneficial gut bacteria and the fermenting bacteria are acting as beneficial entities. This further strengthens our hypothesis that Eu can be considered a beneficial fermented beverage rather than an alcoholic drink (Additional file 6).

Competition index

From the competition index, it was clear that the pathogenic bacteria in the Eu faced intense competition from the excellent gut microbes and fermenting bacteria in the

Eu. Pathogenic bacteria were competing with humans to get their nutrition from the host body and the good gut bacteria of the Eu. Microbial populations were competing with those pathogenic bacteria protecting the human body. This inter-cluster interaction (between the good and bad microbes of Eu) among the Eu microbial population may help to maintain not only the quality of Eu, but also may enhance the upright impact of this drink on the human gut microbiome as well as on the overall health (Additional file 7).

Effect of the compounds of Eu in on the prevention of gastrointestinal cancer

Gastrointestinal cancer is one of the most lethal human cancers, which is a significant unresolved concern in the World (Quazi 2022). The gut is the most intricate part of the human body, comprising a wide range of microbiomes. Dysbiosis of these microbial populations within the GI tract can lead to several diseases, such as obesity, inflammatory bowel syndrome, and colorectal and GI cancer (Quazi 2022). Among all probiotics, lactic acid bacteria can prevent carcinogenic activity through various mechanisms, provide promising results in the treatment of cancer, and are well-known to influence human intestinal health (Jafari-Nasab 2021). Various bacterial constituents from LAB strains can suppress metastasis by modulating the immune system (Patel et al. 2014). The metagenomic analysis of Eu showed mostly the LAB strains. However, the compounds and supposed targets of Eu against GI cancer remain unclear.

In this research, a 'compound-target-disease' network was established. Our studies showed that compounds of Eu were interacting with CES1, CHRM5, CNR2, CTSD, DPP8, DPP9, GGPS1, HIF1A, HSD17B10, MAPK1, NFKB1, PTGS2, RECQ2, S1PR2, S1PR3, SLC6A5, TDP1, TRIM24, and these proteins are involved with gastrointestinal cancer such as stomach cancer, colon cancer, and pancreatic cancer. A compound can be active against multiple target proteins, whereas multiple compounds can modulate a target. Thus, compounds of Eu can work on a polypharmacological level to fight different GI diseases. A crucial immunological feature of GI cancer is chronic inflammation during the over-activation of NF-κB and STAT3 (activator of transcription 3). This over-activation leads to oncogenic proliferation and explosion (Lee et al. 2016). From our in silico studies, we have found that all 13 compounds can target and modulate NF-κB. In addition, several pieces of evidence showed that n-hexadecanoic acid is an anti-inflammatory agent (Aparna et al. 2012).

Another protein regulated by Eu compounds was prostaglandin endoperoxide synthase-2 (PTGS2). PTGS2 has the highest outdegree of 25, followed by NF-κB (an outdegree of 19). The PTGS2 gene codes for the cyclooxygenase enzyme, which converts arachidonic acid to prostaglandins. This can enhance the oncogenic signal. Most of the Eu-derived compounds were found to be interacting with the PTGS2 protein. Thus, they may modulate the expression of PTGS2 protein, preventing the chances of intestinal metaplasia. PTGS2 gene expression can be modulated by *Helicobacter pylori* infection in the gastric mucosa (Maddah et al. 2024). The metagenomic analysis of Eu did not identify *H. pylori* in the Eu microbial population, indicating a positive role of Eu in maintaining a healthy gut system.

Mitogen-activated protein kinases (MAPK) are the serine-threonine kinases that play an active role in cell proliferation and cancer onset. The endogenous formation of reactive oxygen species can trigger the expression of this protein. The network pharmacology study revealed several Eu compounds interacted with the MAPK1, modulating its expression. Furthermore, a previous report on the in vitro antioxidant assay of Eu proved that Eu can reduce oxidative stress (Bhattacharjee et al. 2021). Thus, the antioxidant property of Eu can reduce the expression of MAPK, plummeting the chances of GI oncogenic onset.

The schematic representation of the network is shown in Fig. 5.

Conclusions

Human health is positively regulated with the help of probiotic organisms by enormously stimulating the immune system and inhibiting the growth of pathogens. In the ongoing post-pandemic situation, using probiotics and prebiotics is an alternative strategy against microbial infection. There are plenty of food and beverages conventionally produced from cereals. However, a wide variety of ethnic products have not gotten enough scientific attention as required. In this study, we have analysed the biochemical, nutritional, and network pharmacological aspects of Eu, which has revealed that this ethnic brew has very little alcoholic content but a high protein and free amino acid. This result leads us to think that the Eu may be used as a healthy beverage. Furthermore, the metagenomic analysis identified a considerable population of lactic acid and other fermenting bacteria. Those bacteria are primarily associated with our gastrointestinal tract. Thus, consumption of Eu may also enrich the populace of these good gut microbes in our bodies since it is now a fact that there is a direct association between our gut and brain functionality through the 'gut-brain-axis', an enriched community of good bacteria in our gut can lead to improved cognitive function. Moreover, the presence of lactic acid in Eu has numerous beneficial activities like immune-boosting, lowering cholesterol

Fig. 5 A network analysis of Eu-derived compounds of the sample with a special emphasis on gastrointestinal cancer

levels, and modulating intestinal health. Network pharmacological data support that the consumption of Eu can prevent gastrointestinal cancer. Hence, we may hypothesise that this ethnic brew from the Toto community in West Bengal, India, has the potency to become a healthy drink, improving our overall health and well-being and preventing gastrointestinal cancer. However, the multifaceted nature of the human gut microbiome and the dose-dependent nature of the compounds need further detailed study through in vitro and in vivo analysis to validate these preclinical findings.

Abbreviations

PCR   Polymerase chain reaction
GC–MS   Gas Chromatography–Mass Spectroscopy
LAB   Lactic acid bacteria

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42269-024-01171-9.

Additional file 1 Map of the study area, Totopara, India (*source*: earth.google.com).

Additional file 2 Schematic illustration of the complete 16S metagenomics process

Additional file 3 A network analysis of competition on Eu microbiota.

Additional file 4 A network analysis of Complementation on Eu microbiota

Additional file 5 A table for all the organisms used in the reverse ecology analysis and their corresponding abbreviations used in the heatmap based on reverse ecology

Additional file 6 Heat map on Complementation of Eu microbiota

Additional file 7 Heat map on Competition on Eu microbiota

Author contributions

AS, IS, and SB conceived the idea and did the experimental design. SB and CG collected and prepared the sample for metagenomics. SB, MS, and SR did the nutritional profiling. AS, SB, and IS did the bioinformatics analysis. AS, SB and IS mostly do figures and artwork. All the authors contributed to the manuscript writing and approved it.

Funding

Not applicable.

Availability of data and materials

The authors verify that the data sustaining the findings of this study are presented within the article and its supplementary materials. 16S metagenomic data for the sample has been submitted in NCBI SRA under the BioSample accession number SAMN29515284 with BioProject ID: PRJNA856115.

Code availability

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors in the manuscript were informed about the study and were provided with written consent.

Competing interests

All the authors do not have any competing interests.

Author details

[1]Molecular Cytogenetics Laboratory, Department of Botany, University of North Bengal, Siliguri 734013, India. [2]Plant Biochemistry Laboratory, Department of Botany, University of North Bengal, Siliguri 734013, India. [3]Laboratory of Tea Taxonomy and Ecology, Department of Tea Science, University of North Bengal, Siliguri 734013, India. [4]Biswa Bangla Genome Centre, University of North Bengal, Siliguri, India.

References

Aguirre M, Collins MD (1993) Lactic acid bacteria and human clinical infection. J Appl Bacteriol 75:2

Ahirwar A, Rai A, Sirotiya V, Khandelwal P, Singh G, Jhadav D, Vinayak V (2024) Fermentation of algal biomass for its nutritional value: perspectives and revolutions in the food industry. Environ Technol Rev 13(1):1–28

Aparna V, Dileep KV, Mandal PK, Karthe P, Sadasivan C, Haridas M (2012) Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment. Chem Biol Drug Des 80(3):434–439

Asrani P, Ali A, Tiwari K (2023) Millets as an alternative diet for gluten-sensitive individuals: a critical review on nutritional components, sensitivities and popularity of wheat and millets among consumers. Food Rev Intl 39(6):3370–3399

Bayoï JR, Ndegoue SV, Etoa FX (2021) Traditional processing and quality attributes of "Kounou", a fermented indigenous cereal-based beverage from the northern zone of Cameroon. J Agri Food Res 6:100–209

Bhattacharjee S, Kar P, Sarkar I, Sen A, Ghosh C (2021) Biochemical and microbial profiling establish "Eu"(a traditional fermented beverage of Toto people) as a probiotic health drink. J Ethn Foods 8(1):1–3. https://doi.org/10.1186/s42779-021-00093-5

Bhattacharjee S, Sarkar I, Sen G, Ghosh C, Sen A (2023) Biochemical and Metagenomic sketching of microbial populations in the starter culture of 'Chokot', a rice-based fermented liquor of Rabha Tribe in North Bengal. India Ecol Genet Genom 1(29):100193

Blandino A, Al-Aseeri ME, Pandiella SS, Cantero D, Webb C (2003) Cereal-based fermented foods and beverages. Food Res Int 36(6):527–543

Cao Y, Wang Y, Zheng X, Li F, Bo X (2016) RevEcoR: an R package for the reverse ecology analysis of microbiomes. BMC Bioinformatics 17(1):1–6

Cox DR (1962) Further results on tests of separate families of hypotheses. J R Stat Soc Ser B 24(2):406–424

Das M, Kundu D, Singh J, Rastogi A, Banerjee R (2017) Physiology and biochemistry of indigenous tribal liquor Haria: a state of art. Adv Biotechnol Microbiol 6(2):15

Dridi C, Millette M, Uscanga BR, Salmieri S, Allahdad Z, Lacroix M (2023) Evaluation of the nutritional quality and in vivo digestibility of probiotic beverages enriched with cricket proteins. Food Bioprocess Technol 4:1–9

Du Y, Liu L, Yan W, Li Y, Li Y, Cui K, Zhao H (2023) The anticancer mechanisms of exopolysaccharide from Weissella cibaria D-2 on colorectal cancer via apoptosis induction. Sci Rep 13(1):21117

Georgieva R, Yocheva L, Tserovska L, Zhelezova G, Stefanova N, Atanasova A, Karaivanova E (2015) Antimicrobial activity and antibiotic susceptibility of *Lactobacillus* and *Bifidobacterium* spp. intended for use as starter and probiotic cultures. Biotech Equip 29(1):84–91

Gomes RJ, de Fatima BM, de Freitas RM, Castro-Gómez RJ, Spinosa WA (2018) Acetic acid bacteria in the food industry: systematics, characteristics and applications. Food Technol Biotechnol 56(2):139

Jafari-Nasab T, Khaleghi M, Farsinejad A, Khorrami S (2021) Probiotic potential and anticancer properties of *Pediococcus* sp. isolated from traditional dairy products. Biotechnol Rep 29:e00593. https://doi.org/10.1016/j.btre.2021.e00593

Lee HJ, Park JM, Han YM, Gil HK, Kim J, Chang JY, Hahm KB (2016) The role of chronic inflammation in the development of gastrointestinal cancers: reviewing cancer prevention with natural anti-inflammatory intervention. Expert Rev Gastroenterol Hepatol 10(1):129–139

Maddah R, Molavi Z, Ehymayed HM, Miri F, Shariati P, Veisi M (2024) Identification of shared hub genes and pathways between gastric cancer and Helicobacter pylori infection through bioinformatics analysis. Human Gene 1(39):201237

McCleary BV (2023) Measurement of dietary fiber: which AOAC official method of analysis to use. J AOAC Int 106(4):917–930

Patel PJ, Singh SK, Panaich S, Cardozo L (2014) The aging gut and the role of prebiotics, probiotics, and synbiotics: a review. J Clin Gerontol Geriatri 5(1):3–6

Plummer EW, Salaneck WR, Miller JS (1978) Photoelectron spectra of transition-metal carbonyl complexes: comparison with the spectra of adsorbed CO. Phys Rev B 18(4):1673

Puntillo M, Peralta GH, Bürgi MDM, Huber P, Gaggiotti M, Binetti AG, Vinderola G (2022) Metaprofiling of the bacterial community in sorghum silages inoculated with lactic acid bacteria. J Appl Microbiol 133(4):2375–2389

Quazi S (2022) Anti-cancer activity of human gastrointestinal bacteria. Med Oncol 39(12):1–25

Sarkar I, Kar P, Sen G, Chhetri S, Bhattacharya M, Bhattacharyya S, Sen A (2022) Metagenomic outlooks of microbial dynamics influenced by organic manure in tea garden soils of North Bengal, India. Arch Microbiol 204:1–9

Saxena V, Padhi SK, Jhunjhunwala U (2021) Treatment of domestic sewage and leachate using a moving bed hybrid bioreactor. Environ TechnolInnov 24:101998

Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Ideker T (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 13(11):2498–2504

Singh AK, Rehal J, Kaur A, Jyot G (2015) Enhancement of attributes of cereals by germination and fermentation: a review. Critic Rev Food Sci Nutr 55(11):1575–1589

Stein MI (1948) The thematic apperception test: an introductory manual for its clinical use with adult males. Addison-Wesley. https://doi.org/10.1037/11231-000

Stojanov S, Berlec A, Štrukelj B (2020) The influence of probiotics on the firmicutes/bacteroidetes ratio in the treatment of obesity and inflammatory bowel disease. Microorganisms Phys Rev 11:1715. https://doi.org/10.3390/microorganisms8111715

Tamang JP (2020) History and culture of Indian ethnic fermented foods and beverages. In: Tamang J (ed) Ethnic fermented foods and beverages of India: science history and culture. Springer, Singapore

Teixeira CG, Fusieger A, Milião GL, Martins E, Drider D, Nero LA, de Carvalho AF (2021) Weissella: an emerging bacterium with promising health benefits. Probiotics Antimicrob Proteins 9:1–1

Vinderola CG, Prosello W, Ghiberto D, Reinheimer JA (2000) Viability of probiotics (*Bifidobacterium, Lactobacillus acidophilus* and *Lactobacillus casei*) and non probiotic microflora in Argentinian Fresco cheese. J Dairy Sci 83(9):1905–1911

Wang C, Zhao J, Zhang H, Lee YK, Zhai Q, Chen W (2021) Roles of intestinal bacteroides in human health and diseases. Crit Rev Food Sci Nutr 61(21):3518–3536

Yang X, Yu D, Xue L, Li H, Du J (2020) Probiotics modulate the microbiota–gut–brain axis and improve memory deficits in aged SAMP8 mice. Acta Pharmaceutica Sinica B 10(3):475–487

Zaib S, Hayat A, Khan I (2024) Probiotics and their beneficial health effects. Mini Rev Med Chem 24(1):110–125

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.