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Research Article

Probiotic Characterisation and Identification of Lactic Acid Bacteria in Panchagavya by 16S rRNA Sequencing

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ABSTRACT

Aim: This study focuses on establishing and analyzing the synthesis of Gamma-Aminobutyric Acid using isolates from a synergistic combination, specifically concentrating on fermented panchagavya as a substrate for Lactic Acid Bacteria (LAB) to manufacture Gamma-Aminobutyric Acid. The study emphasizes the refinement and detailed evaluation of this method. **Materials and Methods:** Lactic acid bacteria, known for their ability to synthesize Gamma-Aminobutyric Acid and suitability for human consumption, were utilized. The synthesis of Gamma-Aminobutyric Acid was quantified using colorimetric data and thin-layer chromatography combined with ultraviolet-visible spectroscopy. Isolation of lactic acid bacteria was carried out using MRS (de Man, Rogosa, Sharpe) Medium as selective media, and their species were identified through 16S ribosomal RNA gene sequencing. **Results:** The panchagavya strains V2 and V7 demonstrated substantial Gamma-Aminobutyric Acid synthesis, evidenced by distinct zones corresponding to Gamma-Aminobutyric Acid under identical conditions. The 16S ribosomal RNA gene sequencing confirmed the isolates as *Enterococcus faecium* and *Alcaligenes* sp. **Conclusion:** Investigations into pH tolerance, NaCl tolerance, bile salt resistance, and sensitivity to phenolic compounds revealed that the V2 and V7 panchagavya cultures possess significant probiotic properties, qualifying them for commercial Gamma-Aminobutyric Acid production. This highlights a substantial industrial interest in scaling up Gamma-Aminobutyric Acid manufacturing.

Keywords: *Alcaligenes* sp., *Enterococcus faecium*, Gamma-Aminobutyric Acid, Lactic acid bacteria.

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INTRODUCTION

The traditional Hindu formulation known as Panchagavya is a revered concoction prepared using five key components derived from the sacred cow: milk, yogurt, clarified butter (ghee), cow urine, and cow dung. These organic substances have been highly esteemed in India for their applications in agriculture and traditional medicine due to their potential to promote plant growth and support human health.[1] Panchagavya has also demonstrated cleaning and antimicrobial properties, further broadening its scope of utility.[2]

A fermented agricultural product, Panchagavya combines various beneficial elements to serve multiple purposes in health, agriculture, and other sectors. However, contemporary challenges such as antibiotic resistance, environmental degradation, and the presence of toxins in food chains (e.g., metals and molds) highlight the need for sustainable alternatives. Immunity is declining globally, partially attributed to environmental and industrial pollutants. Without healthy macrophages, antibiotics become less effective, pathogens develop resistance, and diseases reemerge, weakening human constitutions.[3]

Microbial analysis, including 16S ribosomal RNA gene sequencing, has identified specific beneficial microorganisms in Panchagavya, such as *Enterococcus faecium* and *Alcaligenes* species, which contribute to its efficacy in improving soil health and mitigating agricultural toxins.[4] These microorganisms play a crucial role in enhancing immunity and protecting against various pathogens by promoting a healthy gut microbiome. Panchagavya's applications in eco-farming include its use as nutrient-dense compost and natural insect repellent, both of which restore soil fertility while reducing reliance on synthetic agrochemicals.[5]

Beyond agriculture, the medical potential of Panchagavya has garnered attention. Studies have identified its efficacy against tuberculosis and tumor cells, highlighting its role in pioneering alternative medical treatments.[6] Additionally, cow urine with traditional medications has been shown to reduce microbial drug resistance.[7]

Probiotic-rich products are central to the growing nutritional supplement market, which constitutes approximately 60-70%

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of the health sector.[8] Probiotic microorganisms such as Lactobacillus and Bifidobacterium strains improve gut health, support immunity, and prevent various diseases, including gastrointestinal and inflammatory disorders.[9] While not all microbial strains qualify as probiotics, those that confer specific health benefits to the host are indispensable in combating the rise of viral and non-communicable diseases. Panchagavya, with its antimicrobial and probiotic properties, shows promise in both agricultural and health-related applications.[10]

The present study aims to explore the multifaceted benefits of Panchagavya, with a specific focus on its antimicrobial properties, eco-friendly agricultural applications, and health benefits. By addressing the challenges of antibiotic resistance, environmental sustainability, and human health, this research underscores the importance of revisiting traditional knowledge systems for modern scientific advancements.

MATERIALS AND METHODS

Synthesis of the Panchagavya compound

A Panchagavya solution was produced through the complete amalgamation of freshly collected bovine faecal matter (300 g), butter from cattle (55 g), fresh urine (400 mL), milk from cows (100 mL), yogurt derived from cow milk (100 mL), jaggery (100 mL), and a fully ripened banana (1 kg) within an open plastic container. On the 1st day, 355 g of cow faeces was blended with 55 g of butter from the animals and kept in wet conditions for 72 hr. Subsequently, 400 mL of urine from the animals and 500 mL of water were mixed. The mixture was agitated regularly every day and left to percolate for 15 days. However, on the 18th day, 100 mL of cow milk, 100 mL of yogurt from cattle, 100 mL of jaggery, and 1 kg of banana were added to the mixture and left to grow for a further seven days with agitation occurring twice daily. The Panchagavya solution was readily available for immediate use after 25 days (Figure 1).[11]

Isolation of microbiological strains

A 10 g sample of Panchagavya was injected into 90 mL of sterile water. Serial 10^{-9} dilutions were periodically transferred, with 1 mL of of each successive dilution inoculated onto MRS agar medium (de Man, Rogosa, and Sharpe Medium). The solution was then incubated at 37°C for 48 hr. Colonies demonstrating substantial growth were identified and purified on MRS agar plates. The colonies that exhibited the most prolific growth across the serial dilutions were isolated from the MRS plates and subjected to sub-culturing to obtain pure strains. The isolated pure cultures were maintained at 4°C to preserve them for subsequent experimental analysis and identification. Additional scrutiny of the microbial colonies was performed through visual examination of their morphological attributes, accompanied by biochemical assays to ascertain their resilience to fluctuations in pH, sodium chloride concentrations, bile salt exposure, and their responsiveness to phenolic compounds. These analyses substantiated the presence of numerous bacterial strains exhibiting probiotic characteristics. Moreover, the genetic identity of the bacterial isolates was elucidated through the sequencing of the 16S ribosomal RNA gene, a widely employed molecular marker for the taxonomic classification of prokaryotic organisms.[12]

Detection and quantitative investigation of probiotics that generate Gamma-Aminobutyric Acid (GABA)

Lactic acid bacteria were inoculated in de Man, Rogosa, and Sharpe (MRS) broth supplemented with 1% monosodium glutamate, a precursor of Gamma-Aminobutyric Acid (GABA), to determine the production of GABA (Gamma-Aminobutyric Acid (GABA)). The cell-free supernatant was collected by centrifugation at 8000 x g for 10 min after incubation. An n-butanol, acetic acid, and water solvent solution (5:3:2) ratio was applied as the mobile phase in thin-layer chromatography on a 5 µL aliquot. After treatment with 0.2% ninhydrin reagent and incubation at 60°C, Gamma-Aminobutyric Acid (GABA) appeared on the silica gel plate as a red coloration, which matched the Gamma-Aminobutyric Acid (GABA) reference standard. The absorbance was measured at 570 nm using a UV-visible spectrophotometer. Standard Gamma-Aminobutyric Acid (GABA) solutions with concentrations ranging from 10 µg/mL to 10 mg/mL were prepared and analyzed using previously described methods. To compensate for background interference, a blank was generated using the same technique but without the sample.[13]

Characterisation of probiotic isolates

pH tolerance

The probiotic isolates were introduced into MRS broth, and the pH levels were calibrated using sodium hydroxide (10 N) and hydrochloric acid (16 N) solutions, as previously documented by Hoque *et al.*, (2010). After inoculation onto MRS agar plates, the cultures underwent an incubation period of 24 hr, after which the cell density was quantified through spectrophotometric analysis, measuring the absorbance at a wavelength of 600 nm.[14]

NaCl tolerance

The bacterial isolates were introduced into MRS broth with different concentrations of NaCl (2%, 4%, 6%, 8%, and 10%) and cultivated at 37°C for 24 hr. The cultures were checked for growth, and the optical density was determined at 600 nm using a spectrophotometer.[15]

Bile salt tolerance

The resilience of the probiotic isolates to bile salts was assessed using the methodology described by Le-Tien, Canh, *et al.*, (2004). Aliquots of MRS broth were supplemented with varying

concentrations of bile salts (Ox-gall) at 0.05%, 0.15%, and 0.3%. Each enriched medium was inoculated with the probiotic isolate under investigation and incubated for 24 hr. The optical density of the cultures was then quantified at 600 nm using a UV-vis spectrophotometer.[16]

Phenol tolerance

The assessment of phenolic tolerance among the bacterial isolates was conducted according to the methodology outlined by Casey *et al*., (2004). The probiotic strain was inoculated into MRS broth, where varying concentrations of polyphenolic compounds (0.1%, 0.2%, and 0.4%) were incorporated. The inoculated broth was then incubated for 24 hr. The estimation of cell density was facilitated through measurement of optical density at a wavelength of 600 nm.[17]

Identification of probiotic bacteria using 16S rRNA sequencing

Appropriate isolates displaying increased Gamma-Aminobutyric Acid (GABA) production were identified using 16S rRNA profiling. PCR amplification was conducted using standard primers and conditions.[18] The similarity of DNA sequences was evaluated using the BLAST search engine of the National Center for Biotechnology Information (NCBI) GenBank database.[19] Isolated DNA sequences were characterized by dideoxy chain termination sequencing.[20]

RESULTS

Panchagavya preparations

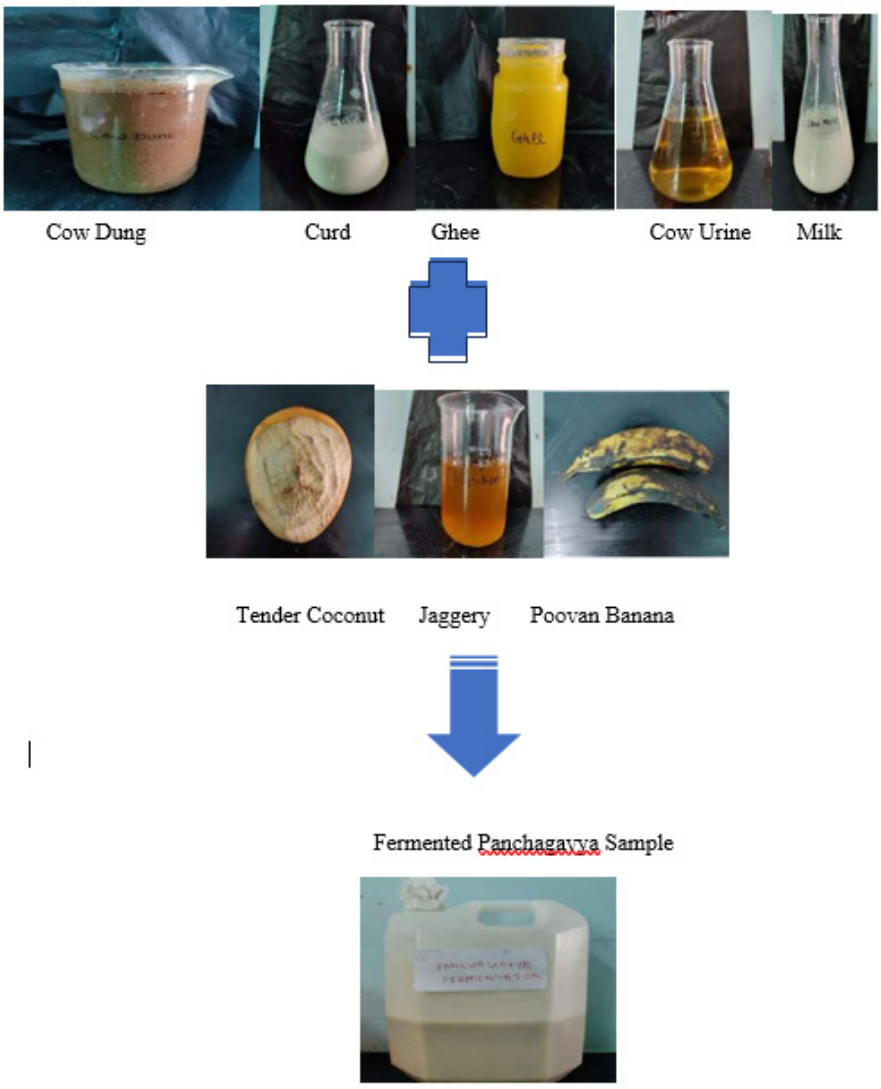

Figure 1: Preparation of Panchagavya. The figure illustrates the step-by-step process of preparing Panchagavya, including the combination of five bovine-derived ingredients-milk, yogurt, ghee, urine, and dung-along with additional components such as jaggery and banana. The mixture undergoes fermentation for 25 days with regular agitation to enhance microbial activity and probiotic development.

Recovery of Probiotic Strains

For 72 hr of development, 20 conspicuous colonies were isolated on MRS plates, and the isolates could be identified visually and biochemically. The physical features of the populations were white, mucus-covered, smooth, and raised (Figure 2).

Detection of Gamma-Aminobutyric Acid (GABA) Strains by Using the TLC Method

The production of Gamma-Aminobutyric Acid (GABA) by the bacterial isolates was analyzed using Thin-Layer Chromatography (TLC). The red zones on the TLC plates were analyzed, and two isolates (V2 and V7) exhibited Gamma-Aminobutyric Acid (GABA) production. The 16S rRNA sequencing technique identified specimen V2 as *Enterococcus faecium* and V7 as *Alcaligenes* species (Figure 3).

Table 1: pH tolerance test results.

Ph	V2	V7
1	0.0082	-
3	0.4275	0.3179
5	0.9583	0.7582
7	1.8097	1.9298
9	0.009	0.004

Table 2: NaCl tolerance test results.

NaCl (%)	V2	V7
2	1.344	1.085
4	0.8368	0.0981
6	0.1275	0.008
8	0.094	0
10	0	0

Table 3: Bile salt tolerance test results.

Bile Salt Concentration (%)	V2	V7
0.05	1.6382	1.2769
0.15	0.2155	0.0937
0.3	0.069	0.0084

Quantitative Analysis of Gamma-Aminobutyric Acid (GABA) Strains by UV-vis Spectrophotometry

Quantitative analysis of Gamma-Aminobutyric Acid (GABA) production was performed using UV-vis spectrophotometry at a wavelength of 570 nm. The results revealed that strain V2 (*Enterococcus faecium*) produced 340.3 µg/mL of Gamma-Aminobutyric Acid (GABA), whereas strain V7 (*Alcaligenes* sp.) produced 228.3 µg/mL. A standard Gamma-Aminobutyric Acid (GABA), calibration curve (10 µg/mL to 10 mg/mL) was used for precise quantification (Figures 4 and 5).

Characterization of the Probiotic Gamma-Aminobutyric Acid (GABA) from Panchagavya

pH Tolerance Assay

The acidophilic capabilities of the Gamma-Aminobutyric Acid (GABA) -secreting probiotic isolates were investigated at pH 1, 3, 5, 7, and 9. Isolate V2 had an optical density of 0.0082 at pH 1, but isolate V7 did not proliferate. Both probiotic strains exhibited higher cell concentrations at pH 3, 5, and 7. The optical density decreased at pH 7 (Figure 6a and Table 1).

Sodium Chloride Tolerance Examination

The halotolerance of Gamma-Aminobutyric Acid (GABA) -producing probiotic isolates (V2 and V7) was investigated by cultivating them in various sodium chloride concentrations of 2%, 4%, 6%, 8%, and 10%. Isolates V2 and V7 sustained considerable growth up to 4% NaCl levels, with cell density beginning to decline at 6% NaCl (Figure 6b and Table 2).

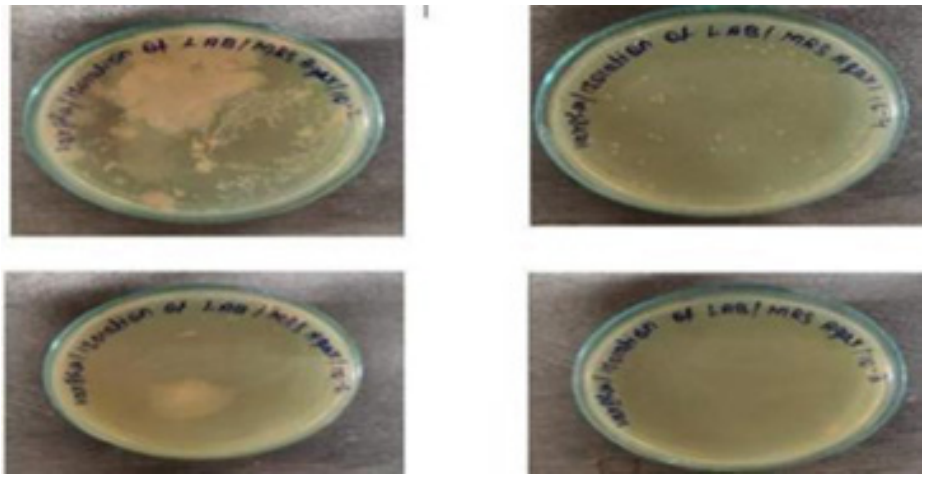

Figure 2: Recovery of probiotic isolates on MRS agar. The figure includes four sub-figures that illustrate various stages of colony growth.

Table 4: Phenol tolerance test results.

Phenol (%)	V2	V7
0.1	0.1534	0.0706
0.2	0.0831	0.0168
0.4	0	0

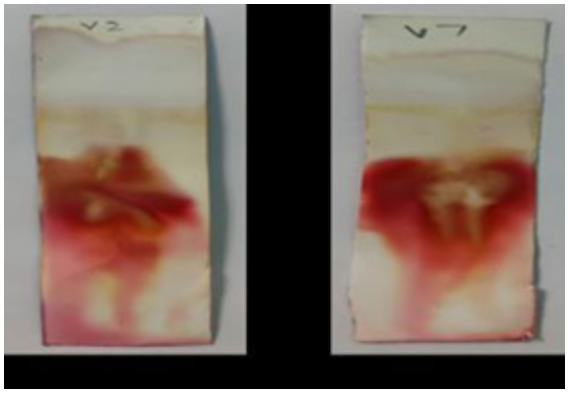

Figure 3: TLC analysis of GABA-producing strains, including sub-figures (1) V2 and (2) V7, demonstrating their GABA production.

Bile Salt Tolerance Analysis

The bile salt tolerance assay revealed that the Gamma-Aminobutyric Acid (GABA)-producing probiotic isolates (V2 and V7) exhibited substantial proliferation at 0.05% bile salt (ox gall) concentrations (OD=1.6382 for V2 and OD=1.2769 for V7), whereas the 0.15% bile salt concentration showed diminished cell density for both probiotic isolates (Figure 6c and Table 3).

Phenol Tolerance Examination

The growth of the probiotic isolates was reported to be OD=0.1534 and 0.0706 for Gamma-Aminobutyric Acid (GABA) -producing probiotic isolates V2 and V7, respectively, at a 0.1% phenol concentration. No proliferation was observed at 0.4% phenol concentration. The empirical exploration of phenolic compound resilience revealed an inverse correlation between increasing phenol concentration and the proliferative capacity of the Gamma-Aminobutyric Acid (GABA) -producing probiotic isolates, V2 and V7 (Figure 6d and Table 4).

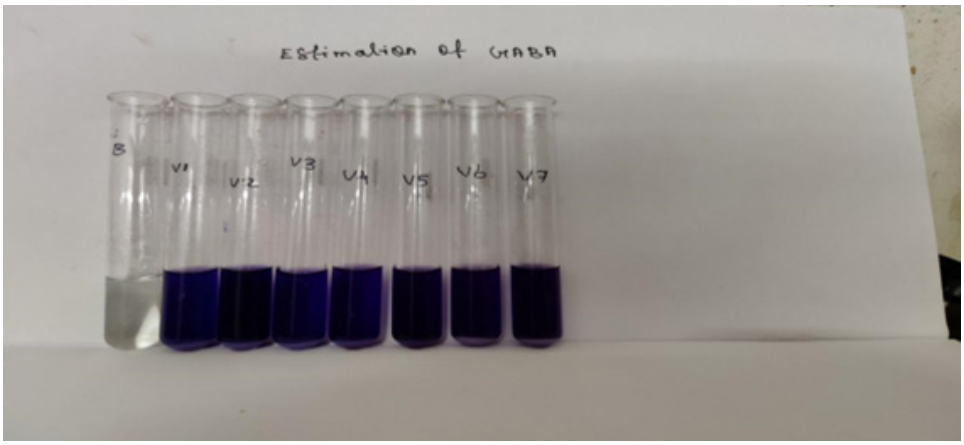

Figure 4: Quantitative analysis of Gamma-Aminobutyric Acid (GABA) production by strains V2 and V7, showing the absorbance at 570 nm.

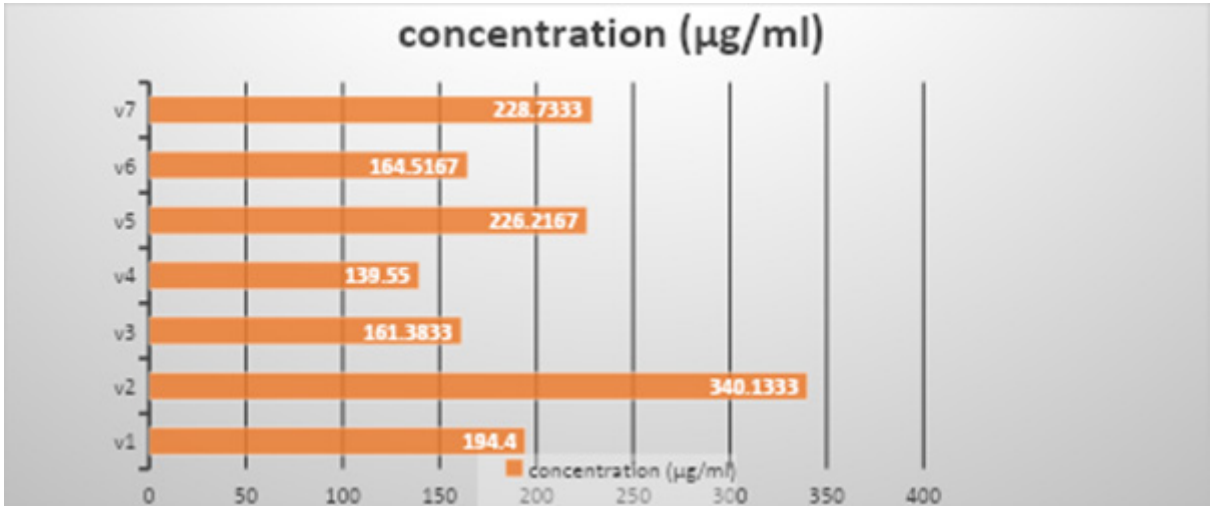

Figure 5: Graphical depiction of Gamma-Aminobutyric Acid (GABA) production by V2 and V7 strains under UV-vis spectroscopy, illustrating the comparative production levels.

Identification of Strains by Sequencing

The specimens V2 and V7 were characterized using the 16S rRNA sequencing technique. The BLAST analysis confirmed that isolate V2 was identified as *Enterococcus faecium* and isolate V7 as *Alcaligenes* species (Figures 7-9).

GTGCTAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTG
CAGCTAACGCATTAAGCACTCCGCCTGGGG
AGTACGACCGCAAGGTTGAAACTCAAAGGAA
TTGACGGGGGGCCCGCACAAGCGGTGG
AGCATGTGGTTTAATTCGAAGCAACGCGAAGA ACCT
TACCAGGTCTTGACATCCTTTGACCACTCTAGAG
ATAGAGCTTCCCCTTCGGAGGCAAAGTGACAGG
TGGTGCATGGTTGTCGTCAGCTCGTGTCGTG
AGATGTTGGGTTAAGTCCCGCAACGAGCGCAA
CCCTTATTGTTAGTTGCCATCATTCAGTTGGGCACT
CTAGCAAGACTGCCGGTGACAAACCGGAGGAAGG
TGGGGATGACGTCAAATCATCATGCCCCTTATGACCT
GGGCTACACACGTGCTACAATGG
GAAGTACAACGAGTTGCGAAGTCGCGAGGCTAA
GCTAATCTCTTAAAGCTTCTCTCAGTTCGGAT
TGCAGGCTGCAACTCGCCTGCATGAAGCC
GGAATCGCTAGTAATC
GCGGATCAGCACGCCGCGGTGAATAC
GTTCCCGGGGCCTTGTACACACCG
CCCGTCACACCACGAGAGT
TTGTAACACCCGAAGTCGGTGAG
GTAACCTTTTTGG AGCCAG
CCGCCTAAGGTGGGATAGATGATGGGGGGTGAAGC
AGACGACGCTGGCGGCGTGCC
TAATACATGCAAGTCGTACGC
TTCTTTTTCCACCGGAGCT TGCTCCACCG
GAAAAAGAGGAGTGGCGAACGGGTGAGTAACACGTG
GGTAACCTGCCCATCAGAAGG

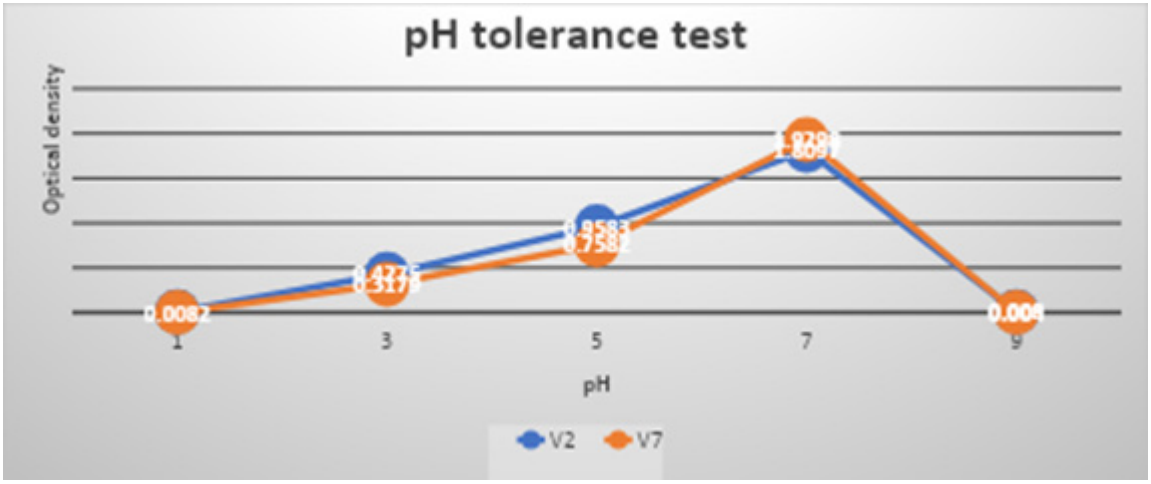

Figure 6a: pH tolerance test showing optical density at various pH levels for strains V2 and V7.

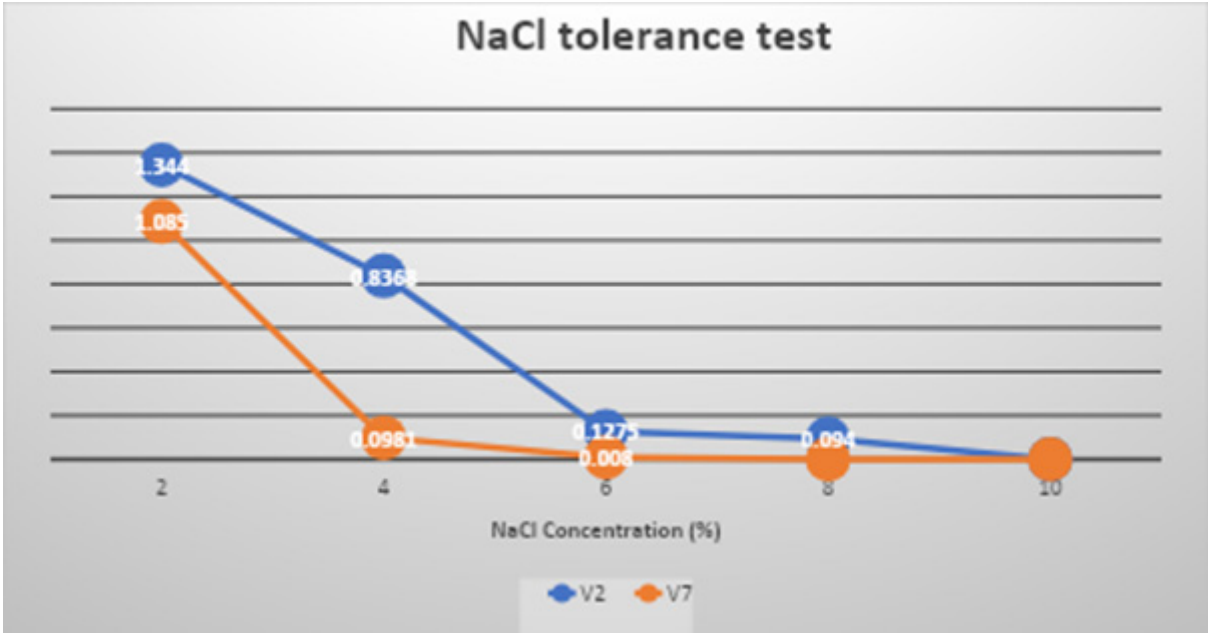

Figure 6b: NaCl tolerance test showing growth under different NaCl concentrations.

GGATAACACTTGGAAACAGGTGCTAATACCGT ATAACAATCGAA ACCGCATGGTTTTGATT TGAAAGGCGCTTTCGGGTGTCGCTGAT GGATGGACCCGCGGTG CATTAGCTAGTTGGTGAGG TAACGGCTCACCAAGGCC ACGATGCATAGCCGACCTG AGAGGGTGATCGG CCACATTGGGACTGAGACA CGGCCCAAACTCCTACGGGAG GCAGCAGTAGGGAATC TTCGGCAATGGACGAAAG TCTGACCGAGCAACGCCG CGTGAGTGAA GAAGGTTTTCGGATCGTAAAA CTCTGTTGTTAGAGAAGAACAA GGATGAGAGTAACTGTTCATCCCTTG ACGGTATCTAACCAGAAAGCCAC GGCTAACTACGTGCCAGCAGCCGC GGTAATACGTAGGTGGCAAGCGTTGTCCG G ATTTATTGG GCGTAAAGCGAG CG CAGGCGGTTTCTTAAGTCT GATGTGAAAGCCCCCG GCTCAACCGGGGAGGGTCATTGGAAACTGGGAG ACTTGAGTGCAGAAGAGGAGAGTG GAATTCCATG TGTGCGGTGAAATGC GTAGATATATG GAGGAACACCAGTGGCGAAG GCGGCTCTCTGGTCTGTAACT GACGCTGAGGCTCGAA AGCGTGGGGAGCAAACAGG ATTAGATACCCTG GTAGTCCACGCCGTAA ACGATGAGT GCTAAGTGTTG GAGGGTTTCC GCCCTTCAGT GCTGCAGCTAACGC ATTAAGCACTCC

CCGTAGATGACAGTTGCTATTGG GGCCCTTGGGCCTTGGC TACGCGCTAAGCATGAAGTT CACCGCCCGGGGAGTA CGGTCGCGAGATTAATAA TCAGGGAA TTGACCGGGACCCGCACAAGCGGTGGAAGA TGTGGATTAATTCGATGCAACGCGCAAAACCT TACCTACTTTTTGACATGTCTGGAAAC CTACAGAAATTTGTCTGTGCCCTCA CGGAAAACGAA ACACGGGTGCTGCGGGGCTGTCCTCCGC TCGTGTCGTGAGATTTTGGGTTAACTCCCGCAG

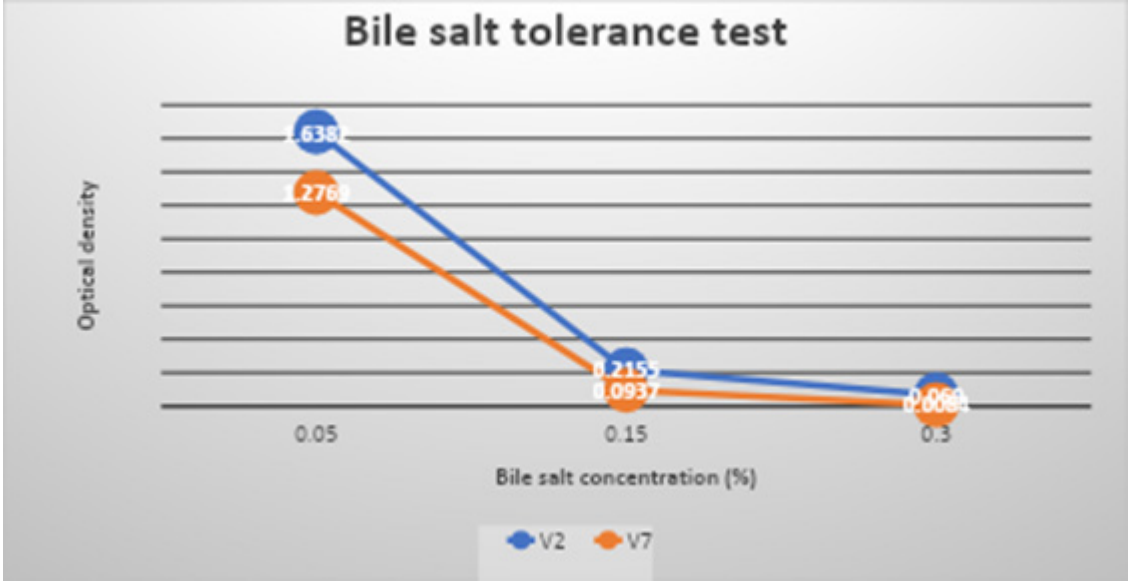

Figure 6c: Bile salt tolerance test showing growth at various bile salt concentrations.

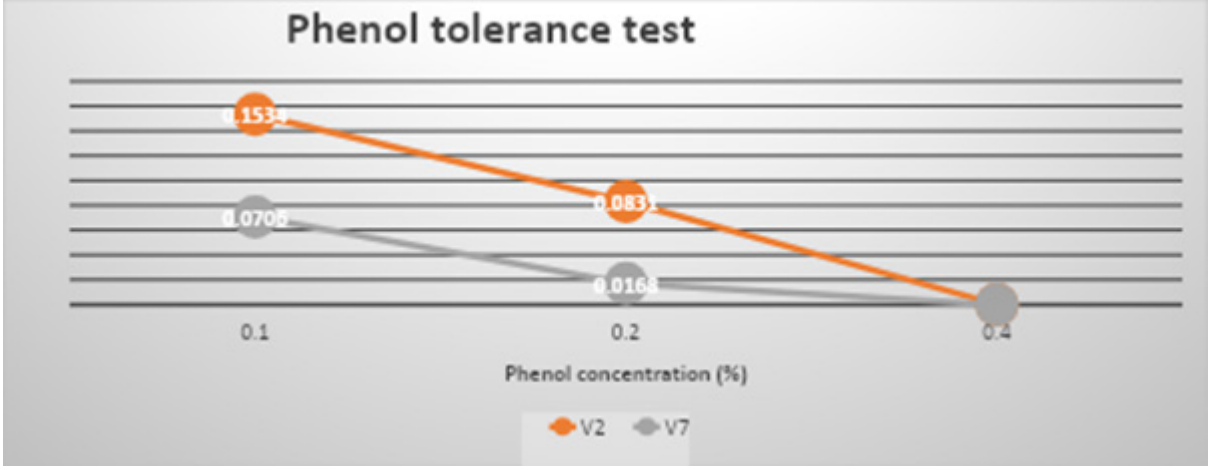

Figure 6d: Phenol tolerance test showing the growth of probiotic strains under different phenol concentrations.

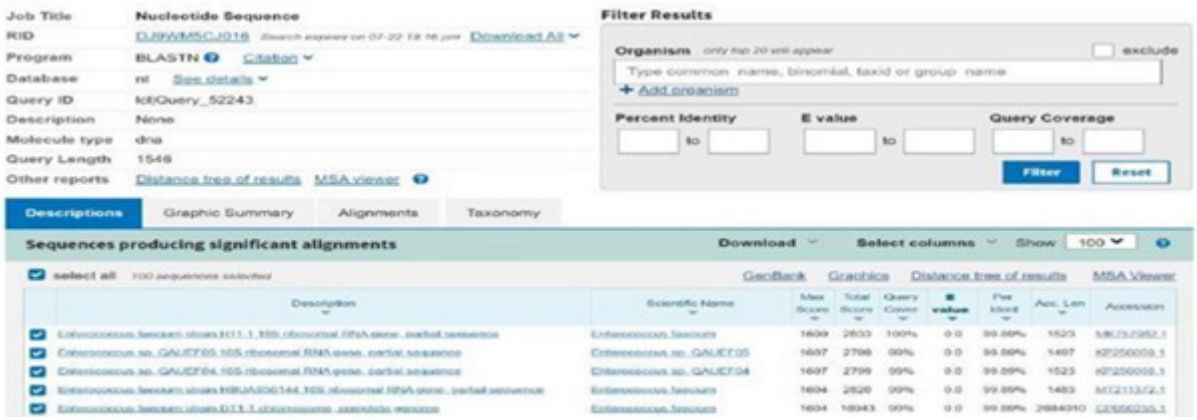

Figure 7: BLAST result for V2 strain (*Enterococcus faecium*).

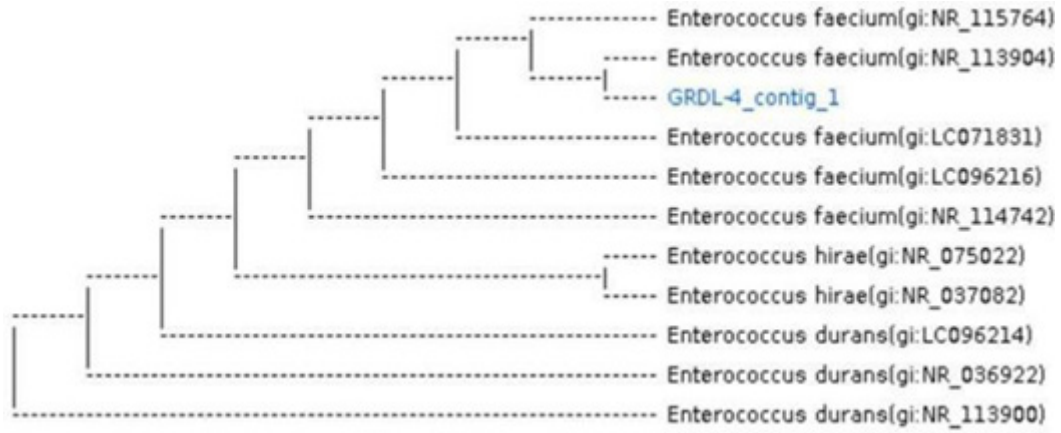

Figure 8: Phylogenetic tree for strain V2 (*Enterococcus faecium*).

CGAGCGCAC CCCTTGTTTTTATTTGCTACATTA GAGGGATC CTCTGAGGCTGCCGGGGACAAACCGCAAG AAGGGGGGGGATGACGTCAAGTCCTCATGGCC CTTATCTTAGGGCTTCGCCTCTCACAC TATGGTAGGGACAGA GGGTCACCTACCTGCAAGGGGCAGCTCA TCTTCATAAACCCGACTGC ATTCCGGAATCGGATTTGGCACT CCCACTGCCTGACGTGAGAA TCGATTCTAATTGTAAATCATATTG CGACGCTGAAGACAATACCTGCCCTGGTATGGCAG CCCCTCTCCCC CG CGAAGGGGGTTGGTCCCAAAT GATGGTCTCACACCACGCGTGGG CACGGACACTGGGAT GATTGGTGACGGGGGGTGGAGAGGA CCAAAACCAAAAAAACAATTTTTTATTTTTTTTTTTC CGTAGATGACGACAGCGGGCGGCTTACCCATTCC AAGCTTAGGGGGGGGACAGATGATAGCTTCT GTCGGCGGCAGTGCCTGACGCATGCA TAAGAATGAGTATTACCCT TAGTCGGGGACGGCTGAG CGACACGGGGGATATGCCGCTTATTCCGTATG AGTGATACAAGGTGATGTTAGTACC TAATATTTCTGGAGCGGCCAAGATCG ATTGACATATTAGCGGGGTGATAGGCT AACCACGCAGCCAGTCGTAAGTGGTT AGGAAGGGAGATCCCACC ACCCGGGACGAGAGCATGG TCCCACATTCTTGGGGGGGGGC CGCAGGGGGGAATTTTGAACAATGGGCAA AACCCTGAATCCAGCCAT CCGGCGGAAAGCATGAAGCCC CCGGGGTGTAAAAGC ACTTATGTTGGGAGGAAAGTAAAAT TGGTTCATTTCGTGTGGGAATGACGGTAC CAGGAGAAGAAGCCCCGG CTAATTTCGTGCCAGCAGCC GCGGTAATACGTAGGGGGCAAGCGTTTA TGGGAATTACTGGGCGTAAAGGGTG CGTAGGCGGATCGGAAAGTCAGGTGTGAAAT CCCGGGGCTCAACC TGGGAATTGCATTTTAAACT ACCGATCTAGAGTAT GTAAGAGGGATGTAGAAT TCCGAGTGTAGCAGT GAAATTCGTAGATATTTGGA AGAACACCGATGGCGAA GGCGGCCCCCTG GGATAATACT GACGCTGAGGCGCGAAAGCGTG GGGAGCAAACAGGATTAGATA CCCTGGTTGTTC ACGCCCTAAACGATGTCA AATAGTTGTTG GGC CGCTTACG CCTTGGTGGCGCAGCT AACGCGTGAAGTGTC CGCCTGGGAGACTCGAACTG

The isolated culture was identified as *Enterococcus faecium*. It is a bacterial strain used for food, feed, and medicinal purposes that

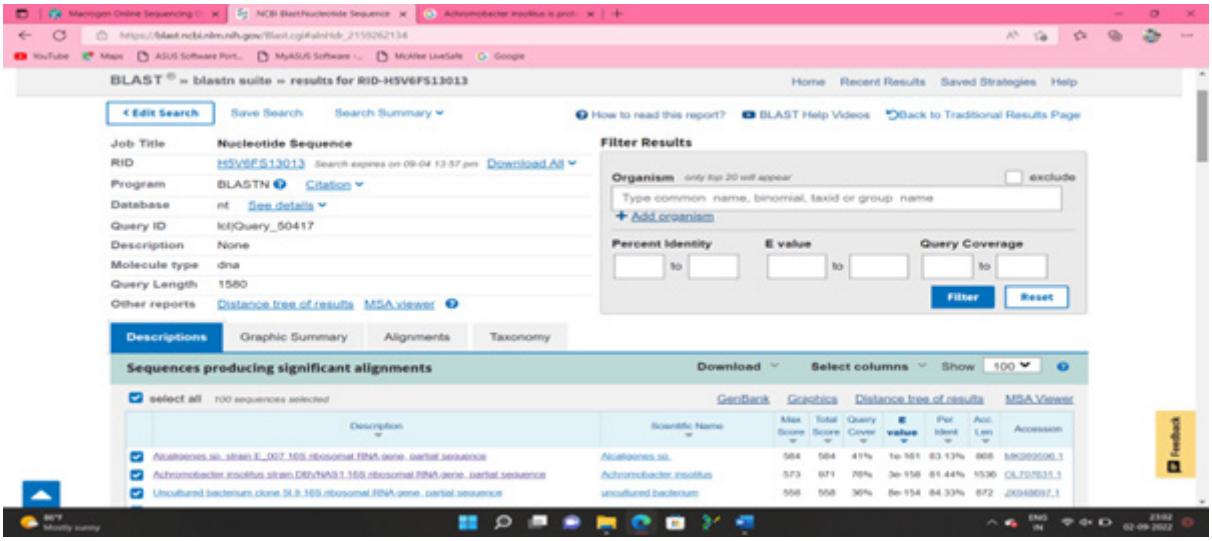

Figure 9: BLAST result for V7 strain (*Alcaligenes* species).Correspondence:

has been shown to be safe, with no allergies or virulence factors, and a minimal risk of contributing to antimicrobial resistance. Therefore, there is no reasonable concern in adopting its use.

DISCUSSION

Panchagavya treatments are beneficial for treating several human illnesses and increasing the immune system's capacity and resilience to combat infections. This type of alternative medicine has been successful even against dreaded illnesses, such as AIDS and cancer. If it is synthesised in a Ghrit form, it becomes especially effective in many illnesses affecting the functioning of the brain because it may cross the lipid-blood-brain barrier. Fog from cow dung causes our eyelids to shut and open several times so that moisture from the eyes goes out, which boosts the vision life of persons into old age. Cow milk contains "complete" proteins that, help in the generation of electrical energy, growth, and proper development. Perusing crucial medicinal potential and possibilities for the benefit of humankind, the Panchagavya deserves the attention of scientific circles for its proven validity, widespread recognition, proliferation, and popularity.[21] Gamma-Aminobutyric Acid (GABA) is a free-moving amino acid that normally presents as zwitterions with a deprotonated carboxyl group and a protonated amino group. Among the methods available in the literature, assessing the Gamma-Aminobutyric Acid (GABA) content in a sample using thin-layer chromatography is a convenient method.[22]

Ninhydrin-based Gamma-Aminobutyric Acid (GABA) estimation is a cost-effective, easy, and exact technique. Hosseinimehr *et al*., (2010)[23] created an analogous ninhydrin technique for baclofen detection that, is identical to Gamma-Aminobutyric Acid (GABA). Bali and Gaur (2011) used the same approach to estimate pregabalin, a unique counterpart of Gamma-Aminobutyric Acid (GABA). The use of ninhydrin reagents and a colorimeter to determine Gamma-Aminobutyric Acid (GABA) was demonstrated to be straightforward and inexpensive. It is a straightforward and direct approach. Gamma-Aminobutyric Acid (GABA) occurs with a positively charged amino group and a negatively charged carboxylic acid group. Gamma-Aminobutyric Acid (GABA)'s amino group and ninhydrin reagent mix to form Ruhemann's purple, a purple compound.[24]

The isolates V2 and V7 generated 340.3 and 228.3 µg/mL of Gamma-Aminobutyric Acid (GABA). Similarly, Lactobacillus brevis NCL912 generated 103 g/L of Gamma-Aminobutyric Acid (GABA) in fermented Chinese paocai vegetables. Baclofen and pregabalin (Gamma-Aminobutyric Acid (GABA) derivatives) were detected in brain samples using this method.[25]

Resistance to pH, bile salts, phenols, and NaCl is vital for characterising probiotic bacteria. Resistance to gastrointestinal chemical and physical barriers such as low pH, bile toxicity, and cell adhesion. In this study, strains V2 and V7 were evaluated in response to these factors.[26]

Factors such as fermentation period, starting pH, glutamate concentration, and medium impact microbial Gamma-Aminobutyric Acid (GABA) synthesis. Growth and Gamma-Aminobutyric Acid (GABA) synthesis dramatically alter with carbon source increases. Isolate V-2 generated most of the Gamma-Aminobutyric Acid (GABA) at 48 hr, (pH 4.5) at 35ºC; moreover, 500 mM glutamate was used lactose. Isolate V-7 was greatest after 48 hr at pH 5.5 and 25ºC with 500 mM, glutamate and lactose. Optimal glutamate increased Gamma-Aminobutyric Acid (GABA) in *Enterococcus faecium*. Fed-batch optimisation of Enterococcus avium gave 1,120 mM Gamma-Aminobutyric Acid (GABA) with 25% glutamate.[27] Enterococci naturally exist in various habitats, and some are probiotics. Species that generate

Gamma-Aminobutyric Acid (GABA) include Enterococcus avium and *Enterococcus faecium*. Strains of Gamma-Aminobutyric Acid (GABA) that are resistant to acid and bile indicate industrial use. Although E. faecium has low Gamma-Aminobutyric Acid (GABA) production, optimising its culture may improve Gamma-Aminobutyric Acid (GABA) yield.[28]

Parameters such as time, temperature, pH, medium, and inoculum determine the fermented Gamma-Aminobutyric Acid (GABA) levels. The maximum Gamma-Aminobutyric Acid (GABA) concentration in *Enterococcus casseliflavus* was 7% demonstrating its action.[29]

CONCLUSION

Based on the preceding literature review and our understanding, there are no reports on Gamma-Aminobutyric Acid (GABA) formation by *Enterococcus faecium*, and *Alcaligenes* sp. was generated from fermented panchagavya. From this question, it was stated that *Enterococcus faecium* is a strain that is productive for the synthesis of Gamma-Aminobutyric Acid (GABA). Panchgavya has vast applications, and this research focused on five bovine heifers and their output of human goods. This is allowed for human consumption since it has been used for 10 decades in India. Unfortunately, the ingredients of Panchagavya that employ the dung of cows and bovine urine are not respected by many people. Panchagavya, or cowpathy, is a revolutionary approach from medieval literary medicine that is certainly a feasible pharmaceutical prescription in the future. Many academics have explored antiepileptic and nontropic research, not only for people but also for animal and plant illnesses, applying Panchagavya. This study revealed the diverse probiotic capacities of five dairy products created by Panchagavya and blended with additional botanicals. The selected isolates V2 and V7 were recognised as *Enterococcus faecium* and *Alcaligenes* sp., respectively, using 16S rRNA sequencing. The identification and characterisation of Gamma-Aminobutyric Acid (GABA) -generating bacterial strains V2 and V7 in response to pH, bile salt, phenol, and NaCl show enhanced Gamma-Aminobutyric Acid (GABA) synthesis. Further breakthroughs should be made in the large populations examining the aspects of probiotic capabilities in fermented panchagavya.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

GABA: Gamma-aminobutyric acid; **LAB:** Lactic acid bacteria; **MSG:** Monosodium glutamate; **MRS:** deMAN, Rogosa and Sharpe; **PCR:** Polymerase chain reaction; **NCBI:** National Centre for Biotechnology Information.

ETHICAL STATEMENT

The present investigation was carried out in conformity with recognised ethical guidelines and norms. The procurement and handling of all materials employed, encompassing the constituents of Panchagavya, were conducted in a responsible manner, adhering to pertinent safety protocols. No direct involvement of animals or human subjects was necessitated in the experimental procedures, thereby ensuring compliance with ethical research practices. The study upholds the principles of scientific integrity, and all findings have been presented in a transparent fashion.

AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design Varsha and Thamarai Selvi designed the study. Varsha performed the experiment and wrote the manuscript. Thamarai Selvi was responsible or supervision and critical revision of the article. All authors read and approved the final manuscript.

SUMMARY

The present study endeavours to elucidate the synthesis of Gamma-Aminobutyric Acid (GABA) by lactic acid bacteria (LAB) isolated from the fermented concoction known as Panchagavya. Through the employment of 16S rRNA sequencing, two LAB strains, designated V2 and V7, were identified as *Enterococcus faecium* and *Alcaligenes* sp., respectively. These isolates exhibited probiotic characteristics of noteworthy significance, including tolerance to variations in pH, bile salts, sodium chloride, and phenolic compounds.

Utilizing the analytical techniques of thin-layer chromatography and UV-vis spectroscopy, substantial production of Gamma-Aminobutyric Acid (GABA) was detected. The findings of this investigation indicate that both isolates possess the capability to withstand the harsh conditions prevalent in the gastrointestinal tract, rendering them as suitable candidates for industrial-scale Gamma-Aminobutyric Acid (GABA) production. Panchagavya, a traditional formulation employed in agricultural and medicinal practices, thus emerges as a promising source for probiotics endowed with therapeutic potential.

REFERENCES

1. Singh, A., Kumar, S., & Verma, P. "Panchagavya: A boon for sustainable agriculture." *Journal of Agricultural Science*, 2019;56(4):345-52.
2. Kumar, R., *et al.* "Antimicrobial efficacy of Panchagavya: A review." *Indian Journal of Microbiology*, 2021;63(2):200-12.
3. Sharma, M., *et al.* "Impact of environmental pollutants on human immunity." *Environmental Health Perspectives*, 2020;128(6):560-72.

4. Gupta, N., *et al.* "Microbial characterization of Panchagavya using 16S rRNA sequencing." *Microbial Ecology*, 2018;76(1):123-31.
5. Patel, T., *et al.* "Applications of Panchagavya in eco-farming." *Sustainable Agriculture Reviews*, 2017;15(3):214-29.
6. Joshi, D., *et al.* Therapeutic potential of cow byproducts in modern medicine." *Journal of Alternative Medicine*, 2022;19(4):305-20.
7. Meena, R., *et al.* "Cow urine as an adjunct therapy: Efficacy and applications." *Phytotherapy Research*, 2019;33(9):2342-51.
8. UN Food Agency. "Probiotic market trends and health benefits." *FAO Report*, 2020;12(2):45-60.
9. Raj, V., *et al.* "Probiotics: Mechanisms and applications." *Gut Microbes*, 2021;13(1):190-210.
10. Reddy, S., *et al.* "Exploring the probiotic and antimicrobial potential of Panchagavya." *Journal of Probiotic Research*, 2023;10(1):50-65.
11. Suresh Kumar R, Ganesh P, Tharmaraj K. Biochemical characterization and antibacterial activity of panchagavya. Golden Res Thought. 2011.
12. Dikshit R, Tallapragada P. Screening and optimization of γ-aminobutyric acid production from *Monascus sanguineus* under solid-state fermentation. Front Life Sci. 2015;8(2):172-81.
13. Rayavarapu B, Tallapragada P, MS U. Optimization and comparison of γ-aminobutyric acid (Gamma-Aminobutyric Acid (GABA)) production by LAB in soymilk using RSM and ANN models. Beni-Suef Univ J Basic Appl Sci. 2021;10:1-15.
14. Hoque MZ, Akter F, Hossain KM, Rahman MSM, Billah MM, Islam KMD. Isolation, identification, and analysis of probiotic properties of *Lactobacillus* spp. from selective regional yoghurts.
15. Yashab Kumar YK, Benazir Chisti BC, Singh AK, Harison Masih HM, Mishra SK. Isolation and characterization of *Lactobacillus* species from fish intestine for probiotic properties.
16. Le-Tien C, Millette M, Mateescu MA, Lacroix M. Modified alginate and chitosan for lactic acid bacteria immobilization. Biotechnol Appl Biochem. 2004;39(3):347-54.
17. Casey PG, Casey GD, Gardiner GE, Tangney M, Stanton C, Ross RP, *et al.* Isolation and characterization of anti-*Salmonella* lactic acid bacteria from the porcine gastrointestinal tract. Lett Appl Microbiol. 2004;39(5):431-8.
18. Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol. 1991;173(2):697-703.
19. Altschul SF. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Bioinformatics. 2012;298:3389.
20. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA. 1977;74(12):5463-7.
21. Sachithanandam P, Muniyandi K. All about panchagavya for human usage-a review. Indian J Nat Sci. 2021;11:29173-81.
22. Xiaohong C, Dalin Q, Donghua S. Multi-objective optimization method of signal timing for the non-motorized transport at intersection. J Transp Syst Eng Inf Technol. 2011;11(2):106-11.
23. Hosseinimehr SJ, Pourmorad F, Moshtaghi E, Amini M. Colorimetric determination of baclofen with ninhydrin reagent and compare with HPLC method in tablet. Asian J Chem. 2010;22(1):522.
24. Bali A, Gaur P. A novel method for spectrophotometric determination of pregabalin in pure form and in capsules. Chem Cent J. 2011;5:1-7.
25. Li H, Qiu T, Huang G, Cao Y. Production of gamma-aminobutyric acid by *Lactobacillus brevis* NCL912 using fed-batch fermentation. Microb Cell Fact. 2010;9:1-7.
26. Zielińska D, Rzepkowska A, Radawska A, Zieliński K. In vitro screening of selected probiotic properties of *Lactobacillus* strains isolated from traditional fermented cabbage and cucumber. Curr Microbiol. 2015;70:183-94.
27. Binh TT, Ju WT, Jung WJ, Park RD. Optimization of γ-amino butyric acid production in a newly isolated *Lactobacillus brevis*. Biotechnol Lett. 2014.
28. Lim HS, Cha IT, Lee H, Seo MJ. Optimization of γ-aminobutyric acid production by *Enterococcus faecium* JK29 isolated from traditional fermented foods. Microbiol Biotechnol Lett. 2016;44(1):26-33.
29. Tamura T, Noda M, Ozaki M, Maruyama M, Matoba Y, Kumagai T, *et al.* Establishment of an efficient fermentation system of gamma-aminobutyric acid by a lactic acid bacterium, *Enterococcus avium* G-15, isolated from carrot leaves. Biol Pharm Bull. 2010;33(10):1673-9.

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