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Original Research Paper

Medical Microbiology

ISOLATION AND CHARACTERIZATION OF LACTIC ACID BACTERIA FROM GABA-PRODUCING PANCHAGAVYA

Varsha M P — Department of Microbiology, Sri Ramakrishna College of Arts and Science for Women, Coimbatore, Tamil Nadu, India

Dr B Thamaraiselvi — Associate Professor, Department of Microbiology, Sri Ramakrishna College of Arts and Science for Women, Coimbatore, Tamil Nadu, India.

ABSTRACT Gamma-aminobutyric acid (GABA) relieves several physiological disorders, including diabetes, hypertension, depression, memory loss, and sleeplessness in humans. Therefore, interest in the commercial production of GABA is gradually rising. Lactic acid bacteria (LAB) have commonly been described as GABA generators and are safe for human intake. The present work intends to optimize and quantify the synthesis of GABA from the isolates derived from the Panchagavya. The probiotic characteristics indicated by different tests like pH tolerance, Nacl tolerance, bile salt tolerance, and phenol tolerance show a considerable presence of probiotics in the isolated cultures (v2 and v7) of the fermented panchagavya. The calorimetric and qualitative investigation of the probiotic culture from fermented Panchagavya two main strains V2 and V7 demonstrated a considerable amount of GABA production under TLC and UV-VIS spectroscopy. Hence, the probiotic isolates obtained from Panchagavya can act as a possible starting culture for the synthesis of GABA in the industrial sector.

KEYWORDS : Gaba, Tlc, Uv-vis Spectroscopy, Panchagavya

INTRODUCTION:

India is a country of traditions with origins in ancient science, closely connecting social rituals and the scientific explanations behind them. In India, a cow is called 'Gaumata' or 'Kamadhenu' because it is nurturing, like a mother. Kamadhenu is the name of the holy cow who believed in completing desired goals. Panchgavya is a storehouse of health advantages and therapeutic characteristics. The Ayurvedic school of medicine has emphasized the importance of employing cow milk, ghee, urine, dung, and curd, each of which is named 'gavya' (i.e., derived from 'Gau' signifies cow) for the treatment of different disorders [1].

Panchagavya has disinfecting and antiseptic effects. Panchagavya is a fermented product in agriculture, along with vermicompost, biopesticides, and biofertilizers. Each Panchagavya element has specific features and usefulness in health, agriculture, and other domains. Scientists and physicians are experiencing issues in current allopathic therapy owing to the various drug resistances in bacteria, the existence of antibiotic residues in the food chain, and/or related allergies and autoimmune illnesses in people and animals. Immunity is dropping dramatically owing to environmental degradation, the use of agrochemicals in agriculture, and pesticides, heavy metals, fungal toxins, etc., in the food chain. Deficient functioning of macrophages leads to the inefficacy of antibiotic treatments, the development of resistance in bacteria, recurring infections, and a potentially impaired immunological condition in an individual.[2]

Panchagavya has several favourable implications In agriculture, including organic farming as excellent-quality natural manure and biopesticides as alternative energy resources with high medicinal properties. Bio-fertilizers and insect repellents derived from cow pee and dung restore micronutrients and fertility of the soil and supply food free from the health concerns of artificial fertilizers and pesticides. No other fertilizer in the world is as inexpensive and harmless as dung fertilizer. Dung and urine can offer vital alternative sources of energy in the form of biogas, fuel, and power. Two US patents have been issued to Indian scientists for demonstrating the boosting characteristics of cow urine, its application in tuberculous patients, and combating tumours, thereby initiating a new era in medical research. Cow urine, coupled with the antibiotics, also reduces the development of resistance in bacteria against the antibiotics [3].

Probiotic food items are recognized as an important element of the functional foods industry, which is rising in sales volume (60–70% of the entire functional food market) and the diversity of goods supplied. According to the FAO/WHO (2006), probiotics are living microorganisms that exert a health effect on the host when supplied in suitable concentrations [4].

MATERIALS AND METHODS

1. Preparation of the panchagavya sample:

Panchagavya solution was created by completely mixing new cow dung (300 g), cow ghee (55g), fresh cow urine (400 ml), cow milk (100 ml), cow milk curd (100 ml), jaggery (100 ml), and ripe banana (1 kg) in an open plastic container. On the first day, 350 g of cow dung was combined with 55 g of cow ghee and maintained for 72 hours, followed by the addition of 400 ml of cow urine and 500 ml of water. The mixture was mixed twice a day and left to ferment for 15 days. On the 18th day, 100 ml cow milk, 100 ml cow curd, 100 ml jaggery and 1Kg banana were added to the mixture and left to ferment for an additional seven days while stirring twice a day. The Panchagavya was ready for usage after a period of 25 days. [5].

2. Isolation of probiotic microorganisms from the panchagavya sample:

10 grams of the Panchagavya sample were dissolved in 90 mL of sterile distilled water. 1 ml of sample was diluted serially for 10–9 dilutions, distributed on MRS medium, and incubated for 48 h at 37 °C. The most prominent colonies were separated and sub-cultured into pure colonies. Pure cultures were kept at 4°C for subsequent investigation [6].

3. Characterization of GABA-generating probiotic isolates:

a. pH tolerance:

Isolates were grown overnight in MRS broth at 37 °C followed by centrifugation at 8000 g for 5 min. Cell pellet was harvested and washed twice in sterile phosphate buffered saline (PBS) pH 7.3 and resuspended in 1 ml of PBS and the strains were further diluted 1:100 in PBS at pH 1, 2, 3 and 4. Samples were then incubated at 37 °C and viable bacterial cells were determined at 0, 60, 120 and 180 min time interval by plating on MRS agar plates. Growth of bacteria was expressed in log10 CFU/ml and survival % of strains was calculated.

b. NaCl Tolerance:

NaCl tolerance was determined by inoculating 100 μl overnight grown culture of the isolates into 900 μl MRS broth

supplemented with 2, 4, 6, 8, 10 and 12% of NaCl and was incubated at 37 °C for 24 h. Tolerance of isolates was analyzed by measuring the absorbance at 600 nm and MRS broth without phenol was taken as reference and further the survival % of strain was calculated.

c. Bile salt tolerance:

Overnight precultures were harvested and resuspended in 5 ml of MRS medium supplemented with 0.3% Oxgall and without as control (Ramos et al., 2013). After inoculation, samples were incubated at 37°C. After a time interval of 0, 1, 2, and 3 h samples were withdrawn and serially diluted using normal saline. Viable cell colonies were enumerated at 0, 1, 2, and 3 h by plating 100 µl of cultures of appropriate dilutions onto MRS agar.

d. Phenol tolerance:

Phenoltolerance was determined by inoculating 100 μl overnight grown culture of the isolates into 900 μl MRS broth supplemented with 0.1–0.5% of phenol and was incubated at 37 °C for 24 h. Tolerance of isolates was analyzed by measuring the absorbance at 600 nm and MRS broth without phenol was taken as reference and further the survival % of strain was calculated.

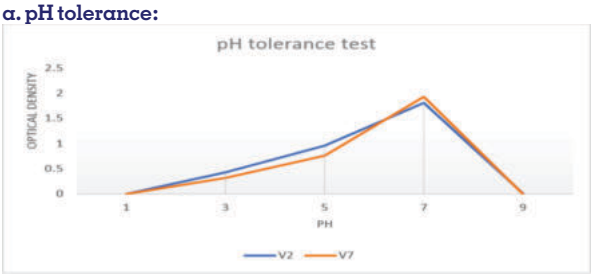
RESULTS:

Isolation Of Probiotic Strains:

In the present investigation, the colonies with typical features were isolated. On MRS plates, the probiotic isolates showed up as glossy, spherical colonies with a creamy-to-off-white hue.

Characterization Of Gaba Producing Probiotic Isolates:

a. pH tolerance:

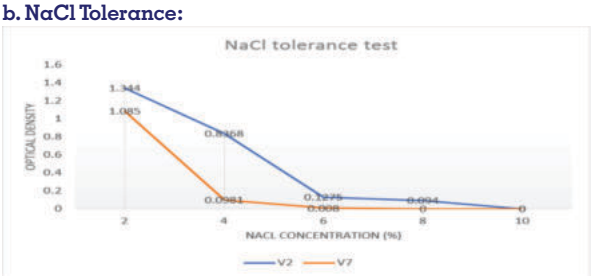


pH tolerance test

Table 1: pH tolerance test

pH	V2	V7
1	0.56	-
3	0.4275	0.332
5	0.9583	0.7582
7	1.17	1.9298
9	0.88	0.56

b. NaCl Tolerance:

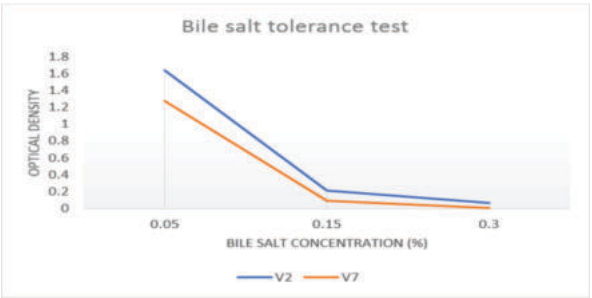


NaCl tolerance test

Table 2: NaCl tolerance test

NaCl (%)	V2	V7
2	1.28	0.22
4	0.456	0.0981
6	0.1275	0.0096
8	0.094	0
10	0	0

c. Bile Salt Tolerance:

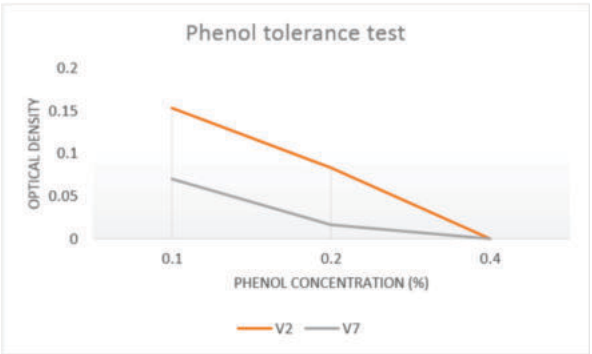


Bile salt tolerance test

Table 3: Bile salt tolerance test

Bile Salt Concentration (%)	V2	V7
0.05	1.6382	2.33
0.15	0.42	0.0937
0.3	0.069	0.0056

d. Phenol tolerance:



Phenol tolerance test

Table 4: Phenol tolerance test

Phenol (%)	V2	V7
0.1	0.1534	0.351
0.2	0.088	0.0168
0.4	0	0

CONCLUSION:

Panchgavya has extensive uses, and this research primarily focused on five cows and Panchagavya human-use goods. Panchagavya is safe for human usage since it has been utilized for 10 decades in India. However the formulations of Panchagavya with cow dung and cow urine are not satisfied by many individuals. Panchagavya, or Cowpathy, is a new approach from ancient literary medicine and undoubtedly a viable therapeutic formulation in the coming years. Many researchers investigated anti-epileptic and nontropic investigations, not only for people but also for animal and plant ailments, employing Panchagavya. Research indicates the numerous probiotic qualities of five cow products, formed Panchagavya and mixed form with other herbs. Further results should be obtained in large population studies, new medication development techniques, and the many possibilities to eliminate diseases. so this study finding analyzes the characteristics of probiotic characteristics from fermented panchagavya.

REFERENCES

[1] Komal K. Bajaj, Vishal Chavhan, Nishikant A. Raut, Shailendra Gurav .,

Panchgavya: A beautiful gift to humanity., Journal of Ayurveda and Integrative Medicine 13 (2022) 100525.
[2] Parkavi Sachithanandam, Kokila Muniyandi, Indian Journal of Natural Sciences, Vol.11 / Issue 64 / February / 2021, International Bimonthly, ISSN: 0976 – 0997.
[3] K DHAMA1, RAJESH RATHORE. RS CHAUHAN AND SIMMI TOMAR., PANCHGAVYA (COWPATHY): AN OVERVIEW, International Journal of Cow Science, 1(1): 1–15.
[4] García-Ruiz, Almudena, et al., "Assessment of Probiotic Properties in Lactic Acid Bacteria Isolated from Wine." Food Microbiology, vol. 44, 2014, pp. 220-225, https://doi.org/10.1016/j.fm.2014.06.015. Accessed December 12, 2023.
[5] Suresh Kumar, R*, Ganesh, P, Tharmaraj, K ; BIOCHEMICAL CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF PANCHAGAVYA, Golden Research THoughts 2, Volume 1, Issue V/Nov 2011.
[6] Dikshit R, Tallapragada P. Screening and optimization of □-aminobutyric acid synthesis from Monascus sanguineus under solid-state fermentation, Frontiers in Life Science, 2015;8(2):172–81. https://doi.org/10.1080/ 21553769. 2015.1028654.
[7] Hoque MZ, Akter F, Hossain KM, Rahman MS, Billah MM, and and Islam KM. Isolation, identification, and investigation of probiotic characteristics of Lactobacillus spp. from chosen regional yoghurts. World J Dairy Food Sci. 2010;5(1):39-46.
[8] Kumar YA, Chisti BE, Singh AK, Masih HA, and Mishra SK. Isolation and characterization of Lactobacillus species from fish gut for probiotic activities. International Journal of Pharma and Bio Sciences. 2013;4(1):11–21.
[9] Le Tien C., Millette M., Mateescu M.A., and Lacroix M., Modified alginate and chitosan for lactic acid bacteria immobilization, Biotechnol. Appl. Biochem. 39 (2004) 347–354.
[10] Casey P.G., Casey G.D., Gardiner G.E., Tangney M., Stanton C., Ross R.P., Hill C., Fitzgerald G.F., Isolation and characterization of anti-Salmonella lactic acid bacteria from the pig gastrointestinal tract, Lett. AppL. Microbiol. 39 (2004) 431–438.