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MICROBIAL ANALYSIS AND IDENTIFICATION OF PANCHAGAVYA MICROBIOTA

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Abstract– Panchagavya is a fermented product made from a mixture of five key components obtained from cows: urine, milk, ghee (Clarified butter), curd, and dung. Furthermore, investigations demonstrated the existence of various beneficial microorganisms in panchagavya, including Actinomycetes, bacteria, and fungus, which play an important role in crop development and soil production. The proteolytic characteristics of Panchagavya were studied in this work utilizing a commercially available Skim Milk Agar (SMA). ZBDS17, a proteolytic bacterium, was successfully isolated. Using 16s rDNA sequencing, it was discovered that ZBDS17 belongs to the *Bacillus* spp. biofertilizer group. Panchagavya pro-agricultural role was validated by this observation. The current research contains original data, and it is critical to establish a new research field dedicated to revealing hidden or dormant Vedic biotechnological principles such as Panchagavya.

INTRODUCTION

Panchagavya means "mixture of five indigenous cow products," which comprises dung, urine, milk, curd, and ghee (Clarified butter). It has traditionally been employed in many ancient Indian health and agriculture practices. Fermented Panchagavya is an organic liquid concoction recorded in ancient Indian literature such as Vrikshayurveda, which describes beneficial plant cultivation practices (Natarajan, 2002). It promotes growth and immunity while also reducing the occurrence of common ailments (Vallimayil *et al.*, 2012). Panchagavya is associated with beneficial microorganisms that promote plant growth by solubilizing phosphate and zinc oxide, producing siderophores and plant growth promoting substances, and increasing plant resistance to various environmental stresses. The beneficial effects of panchagavya, a biodynamic preparation, on various crops have been reported to significantly increase cereal and vegetable crop yield. Panchagavya improves plant tolerance to pests, pathogens, salinity, and drought by developing resistance and increasing soil fertility, nutrients, plant growth, and metabolic shift. These contain macro- and micronutrients, amino acids, and growth-promoting chemicals like as indole acetic acid, gibberellins, and helpful microorganisms, among other things. Panchagavya has been shown to have pro-agricultural action (biocontrol, biofertilizer, growth enhancer, and so on), as well as pharmaceutical value, growth stimulating activity, probiotic and antibacterial potential. Their formulations have been found to have beneficial impacts on a variety of crops. The natural preparation of Panchagavya plays a multifaceted role in plant growth and soil fertility improvement by supplying nutrients, amino acids, vitamins, plant growth-promoting substances, and beneficial microorganisms. It improves plant growth as well as plant defense against various biotic and abiotic environmental stresses.

Panchagavya improves soil fertility and health in addition to improving plant growth and health by increasing soil microbial activity, organic matter decomposition, soil-borne disease suppression, and soil available nutrients (Kumar *et al.*, 2020). Soil microorganisms are crucial to soil fertility, not only because of their ability to carry out biochemical transformations, but also because they serve as a source of mineral nutrients sink. Several microorganisms have the ability to boost crop growth and improve crop health. Several microorganisms have the ability to boost crop

growth and improve crop health. (Murugalatha *et al.,* 2018). Pikovskaya (1948) published the first systematic investigation on phosphate solubilization, which demonstrated the dissolution of tricalcium phosphate by pure culture of 'Bacterium p'.

In acidic, neutral, and alkaline culture medium, isolates dissolved tricalcium phosphate to a significant degree, but rock phosphate was absorbed to a lesser level than TCP (Muromtsev, 1958). By de phosphorylating, soil microorganisms play an important role in increasing plant phosphorus availability. Phosphate-containing organic compounds, as well as by provoking favorable changes in soil reaction and soil micro-environment, resulting in the solubilization of inorganic phosphates. Furthermore, organic acids and humic acid substances, which are primarily produced during organic matter decomposition, form complexes with calcium, iron, and aluminium compounds, reducing phosphorus fixation and indirectly increasing its availability.

Various research groups have isolated Phosphate Solubilizing microorganisms from crop plant soils and rhizosphere in recent years (Halder *et al.,* 1990; Krishna Raj 1990; llmer and Schinner, 1992; Maheshkumar, 1997). The production of organic acids is one of the mechanisms used by these organisms to solubilize the insoluble mineral phosphate (Gaur, 1990). Beneficial effective microbes (EMOs) of lactic acid bacteria, yeast, actinomycetes, photosynthetic bacteria, Azotobacter, Azospirillum, and Phosphobacterium, as well as some fungi, were found in Panchagavya, which are known to improve soil health, crop growth, and yield (Xu and Xu, 2000). Fermented liquid organic fertilizers prepared from cow dung, urine, leguminous leaves, or vermiwash are effective in rapid soil fertility buildup due to increased activity of soil micro-flora and fauna (Yadav and Mcwade, 2004).

Soil microorganisms are crucial to soil fertility, not only because of their ability to carry out biochemical transformations, but also because they serve as a source of mineral nutrients sink. Several microorganisms have the ability to boost crop growth and improve crop health. Spraying these formulations biodynamical has considerably increased grain and vegetable yields. These preparations have been shown to contain naturally occurring beneficial microorganisms, primarily bacteria, yeast, actinomycetes, and some fungi. There are limited findings on the isolation and characterization of beneficial properties of bacteria found in biodynamic preparations. There are also few studies employing molecular approaches to reliably identify the beneficial bacteria in biodynamic treatments. As a result, an attempt was made in this study to identify and molecularly characterize beneficial proteolytic bacteria from Panchagavya.

MATERIALS AND METHODS

Isolation and identification of proteolytic bacterial isolates from Panchagavya

In the laboratory, panchagavya were prepared and proteolytic bacteria were isolated and characterized. The isolates were then streaked and confirmed on Skim Milk agar plates for 24 hours at room temperature. The proteolytic organisms were indicated by a clear zone of inhibition on the SMA. It was streaked on fresh skim milk agar plate to obtain the pure culture based on biochemical and morphological characteristics.

Molecular identification (16s rRNA) of proteolytic isolates

Genomic DNA was isolated from overnight grown cultures in Minimal Salt Broth medium, by cetyltrimethyl ammonium bromide method (Ausubel *et al.*, 1999).

1. DNA was isolated from the culture and its quality was evaluated on 1.0 % agarose gel.
2. A single band of high-molecular weight DNA has been observed.
3. Fragment of 16S rRNA gene was amplified by 16SrRNA-F and 16SrRNA-R primers.
4. A single discrete PCR amplicon band of 1500 bp was observed when resolved on agarose gel.
5. The PCR amplicon was purified to remove contaminants.
6. Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with 16SrRNA-F and 16SrRNA-R primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer.
7. Consensus sequence of 16S rRNA gene was generated from forward and reverse sequence data using aligner software.
8. The 16S rRNA gene sequence was used to carry out BLAST with the 'nr' database of NCBI GenBank database.
9. Based on maximum identity score first ten

sequences were selected and aligned using multiple alignment software program Clustal W.

10. Distance matrix and Phylogenetic tree was constructed using MEGA 10.

RESULTS AND DISCUSSION

All the isolation and analysis methods used for microbial analysis of Panchagavya were found effective against different parameters. The current study used a culture-based method to examine the microbial diversity of Panchagavya samples after the 21st day of preparation, as several microorganisms, including bacteria, fungi, beneficial and proteolytic organisms were found in panchagavya.

The microbial loads increased on 21st day of fermentation in the study. Similar findings were reported by Maheshwari *et al.* (2007). In microbiological study, Lactobacillus (22 x 106 cfu/ml), Methylotrophs (5 x 10^3 cfu/ml), *Pseudomonas* (45 x 10^3 cfu/ml), total anaerobes (11.5 x 10^4 cfu/ml), bacteria (35- 42 x10^9 cfu/ml), fungi (13 -16.5 x 10 cfu/ml), actinomycetes (6- 9 x 10^2 cfu/ml), yeast (2- 22.5x 105 cfu/ml) found in Panchagavya. The emergence of beneficial bacteria from Panchagavya in the rhizosphere as a result of panchagavya application improves plant growth, agricultural yield, and reduces plant diseases. *Pseudomonas* and saprophytic yeasts were found in the Panchagavya, which may have contributed to plant protection because *Pseudomonas* on plant surfaces has been found to induce the production of pathogenesis-related protein, siderophores, antibiotics, and HCN in groundnut and rice. As a result, it could be employed as a biocontrol agent (Meena *et al.*, 2000)

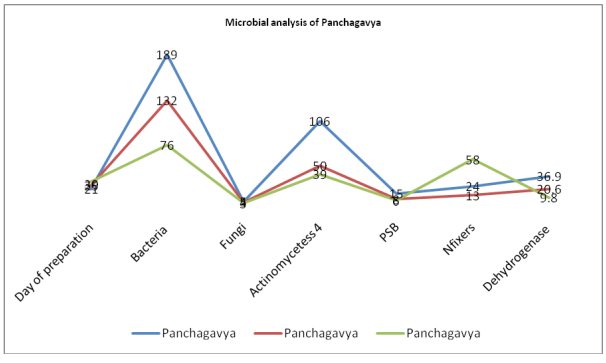

Table 1. Microbial analysis of Panchagavya.

Sl. No	Ingredient	Incubation Time (Day of prep)	Bacteria 10^6 cfu ml-1	Fungi 10^2 cfu ml-1	Actinomycetes 10^4 cfu ml-1	PS-NF 10^5 cfu ml-1		Dehydrogenase µg TPF $g^{-1}day^{-1}$
1	Panchagavya	21	189	05	106	15	24	36.9
2	Panchagavya	25	132	04	50	08	13	20.6
3	Panchagavya	30	76	03	39	6	58	9.8

Molecular identification

Proteolytic bacteria hydrolyze casein and produces nitrogenous components which are indicated as a clear zone on SMA which is standard procedure (Downes *et al.*, 2001).One of the proteolytic bacterial colonies of ZBDS 17 from SMA was selected for the 16s rDNA based molecular identification.

The 16s rDNA gene based analysis in the Phylogenetic tree showed that ZBDS17 isolate was clustered and identified as ***Bacillus australimaris*** and submitted in the database of the National center for Biotechnology with accession no.MW560472.

Using the Maximum Likelihood approach and the Kimura 2-parameter model, the evolutionary history was inferred (Kimura, 1980) the highest log probability tree (-1977.49) is shown.

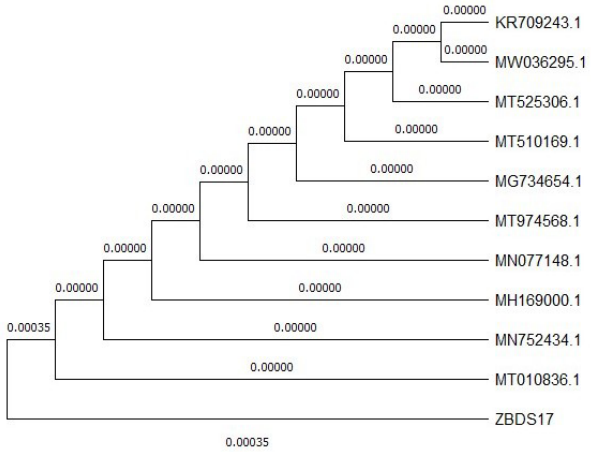

Fig. 1. The inferred Phylogenetic tree showing the proteolytic bacterial isolate ZBDS-17 including reference strain.

The initial tree(s) for the heuristic search were automatically generated by applying the Neighbor-Join and BioNJ algorithms to a matrix of pair wise distances calculated using the Maximum Composite Likelihood (MCL) technique, and then picking the topology with the best log likelihood value. The branch lengths are measured in the number of

Table 2. Estimates of Evolutionary Divergence between Sequences

Distance Matrix											
ZBDS17		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
MT010836.1	0.001		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MN752434.1	0.001	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MH169000.1	0.001	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000
MNO77148.1	0.001	0.000	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000
MT974568.1	0.001	0.000	0.000	0.000	0.000		0.000	0.000	0.000	0.000	0.000
MG734654.1	0.001	0.000	0.000	0.000	0.000	0.000		0.000	0.000	0.000	0.000
MT510169.1	0.001	0.000	0.000	0.000	0.000	0.000	0.000		0.000	0.000	0.000
MT525306.1	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.000	0.000
KR709243.1	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.000
MW036295.1	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	

substitutions per site, and the tree is depicted to scale. There were 11 nucleotide sequences in this study. The first+ second+third+noncoding codon locations were included. The total number of places in the final dataset was 1420. MEGA X was used to undertake evolutionary analysis (Kumar *et al.,* 2018).

The numbers of base substitutions per site from between sequences are shown. Standard error estimate(s) are shown above the diagonal. Analyses were conducted using the Maximum Composite Likelihood model (Kimura, 1980). This analysis involved 11 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1420 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar *et al.,* 2018). A research finding suggests that inoculation of soil or seeds with panchagavya formulations exhibited good plant growth and development without any harmful chemicals (Alori *et al.,* 2012). Bacillus species treated plants showed high growth surveillance easily in soil (Bhutani *et al.,* 2018). Dehydrogenase activity was more on 21st day indicating that panchagavya samples had higher species diversity and abundance which are in agreement with findings of (Chandrakala, 2008). This is likely to provide insight on an array of subjects. Comparable proportions of microbial groups and their relative distribution throughout a period of time and space.

To replace chemical fertilizers with organic sources in organic farming, the quantity required per hectare shall be 37 to 50 tonnes per hectare calculated on the basis of the NPK content. However, based on microbial content, 50 kg cow dung per ha (in split doses of 25 kg twice) is required as the enzymes, especially when the amendment is at its highest dose.

The response was most likely caused by the increased microbial biomass produced in the system (Sumit Pal and Neelam Patel, 2020).

CONCLUSION

Excessive use of inorganic pesticides and fertilizers to boost agricultural productivity have caused severe damage to the environment soil and human health in today's world. Only by adopting new agricultural methods can the negative effects of and these pesticides be avoided. In this context, it is critical to establish an environmentally benign and long-term approach for increasing agricultural productivity and soil fertility. Panchagavya, an organic-based plant growth booster, is the finest option for maintaining sustainable agricultural production without compromising the natural ecosystem. By providing minerals, amino acids, vitamins, growth regulators, and helpful microbes, it has the ability to boost plant growth. It not only improves plant development, but it also improves the plant's resistance to numerous environmental stresses. Panchagavya may use this massive apparatus to his advantage.

The rising concern for environmental safety, as well as the global demand for pesticide residue-free food has sparked a significant interest in crop production utilizing environmentally friendly solutions. As a result, it is vital to employ eco friendly and natural goods such as Panchagavya to create chemical residue free food crops, and it may therefore play a significant part in organic farming. The evaluation of enzyme activity and microorganisms demonstrated that the quality of panchagavya-based fertilizers has enhanced, as has the potential of microorganisms present in the soil

where it was implemented. As a result, the involvement of microorganisms is critical for long-term organic agricultural sustainability.

A proteolytic bacteria isolated from Panchagavya was genotypically identified as belonging to *Bacillus* spp, which are well recognized for their biofertilizer characteristics. Panchagavya present microbiological investigation produced qualitative information on its biofertilizer potential. However, a thorough investigation of the microbial biodiversity associated with Panchagavya pro agricultural and medicinal value is required. The biotechnological understanding of Panchagavya in Ancient writings may be investigated by this research.

Author's contribution

Conceptualization and designing of the research work (H.B. BABALAD and G. SREENIVASALU); Execution of field/lab experiments and data collection (DEEPA & SHEETAL); Analysis of data and interpretation (DEEPA); Preparation of manuscript (DEEPA).

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Conflict of interests

We wish to confirm that there are no known conflicts of interest associated with this publication.

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