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Original Research

Unveiling the Bacterial Communities and Its Potential Agricultural Applications from Organic Manure (Panchagavya) Using Targeted Amplicon Analysis

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Abstract

Due to the alarming effect of synthetic and chemical-based agriculture on human health and the environment, organic farming has been receiving increasing attention around the world. Panchagavya (PG) and farmyard manure play crucial roles in organic farming's nutrient management. Despite its known potential in crop applications, Panchagavya microbial profile has yet to be mapped. The aim of this study is to unveil the succession of bacterial communities in Panchagavya during the fermentation process using targeted amplicon analysis. The results revealed that 45 phyla, 92 classes, 168 orders, 333 families, 899 genera, and 2026 species were obtained from all samples, along with 5144 OTUs. *Proteobacteria* (36.61-70.98%) *and Bacteroidetes* (14.59-23.81%) were found in greater abundance in all samples, with *Proteobacteria* being the most common in all samples, followed by *Bacteroidetes* and *Firmicutes* (12.67-29.58%). *Pseudomonas* was a dominant member of the fermentation process, along with *Romboutsia, Paeniclostridium, Clostridium, Enterococcus, Lactobacillus*, *Bifidobacterium*, and *Ruminococcus*. The findings indicate that Panchagavya demonstrates effectiveness as a fertilizer due to its inclusion of advantageous microorganisms and growth stimulants for plants. Furthermore, some bacterial members that have not yet been characterized for any role in the soil and plant systems, urging a comprehensive investigation into the potential functions for future agricultural applications and practices.

Keywords: Panchagavya, liquid manure, metagenomics, bacterial communities, organic farming

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Introduction

Intensive agriculture using chemical fertilizers has, without a doubt, resulted in a significant increase in farm commodity productivity; however, the negative effects of these chemicals are clearly visible on soil structure, microflora, water quality, food, and fodder. As a result, the current global scenario emphasizes the importance of adopting eco-friendly agricultural practices for long-term and high-quality food production [1]. Furthermore, to address the issue of declining soil fertility caused by synthetic fertilizers and pesticides, it is urgently necessary to establish a viable solution for nutrient maintenance in soil by effective microorganisms utilizing the organic resources for agricultural crops [2, 3]. Sustainable environmentally friendly farming practices, such as organic farming, forego the use of synthetic fertilizers and pesticides in lieu of the use of biofertilizers, which enhance and maintain the soil's nutrient levels [4]. In addition, it increases the diversity of microbes that reside within the soil and promote the development of microbes that are beneficial to the plant and support it to resist disease [5].

For organic food production, farmers frequently turn to locally available organic sources of nutrients such as organic manures, enriched manures, vermicompost, green manures, and liquid organic manures such as Beejamruth, Jeevamruth, Amruthpani, and Panchagavya, and others. Among these liquid organic manures, "Panchagavya," a traditional practice that protects plants and soil microorganisms while also increasing plant productivity, is the most widely practiced and acknowledged [6]. Panchagavya (PG) is a traditional fermented organic product that is widely used to grow a variety of agricultural and horticultural crops [7-9]. It is made by combining five major cow by-products: dung, urine, milk, curd, ghee, and other ingredients. Such bio-stimulants contains distinct macro and micronutrients, amino acids, and growth-promoting substances, in addition to beneficial microorganisms that boost in the production of high-quality crop yields [2, 10]. To improve soil fertility, earthworm quality, and crop health, this bioformulation can be applied as a liquid manure at minimal expense, and it has been shown to increase plant growth and nutrient uptake while reducing biotic and abiotic stresses in crop plants. [11]. In addition, the main inoculants of PG including cow dung and cow urine are excellent sources of energy for the production of biogas and electricity from renewable sources [12].

Assessing microbial diversity in different agroecosystems; identifying microbial cultures for rapid degradation of agricultural wastes; and the role of microorganisms in greenhouse gas mitigation are some of the areas that received the great attention of agricultural microbiologists [6]. Beneficial microorganisms associated with PG are known to promote plant growth through the solubilization of phosphate and zinc oxide, as well as the production of siderophore and plant growth promoting substances, as well as increasing plant resistance to a wide range of environmental stresses [5]. It has also been documented that the beneficial effects of this biodynamic preparation on various crops have resulted in a significant increase in the yield of cereal and vegetable crops [10, 13]. In addition to substances and nutrients that stimulate plant growth, PG also contains a wide variety of effective beneficial microorganisms (EMO), such as *Azospirillum*, *Azotobacter, Pseudomonas, Lactobacillus, Rhodopsuedomonas,* and *Aspergillus*, as well as ammonifiers, nitrifiers, and phosphobacteria. [14].

The effective microorganisms in PG are a mixed culture of naturally occurring beneficial microbes, primarily lactic acid bacteria (*Lactobacillus*), yeast (*Saccharomyces*), actinomycetes (*Streptomyces*), photosynthetic bacteria (*Rhodopsuedomonas*), and certain fungi (*Aspergillus, Penicillium,* etc.) [5]. The beneficial microbes in PG also contains chemolithotrops and autotrophic nitrifies (Ammonifiers and nitrifiers), which colonize on the leaves and increase the ammonia uptake as well as the total N supply [15]. A decrease in the pH of PG was observed at 30-day fermentation; this may could be attributed to the presence of *Lactobacillus* in PG, which produced more organic acid during fermentation process [1]. Furthermore, PG not only increases the number of beneficial microbes in the environment, nonetheless, it also acts as a catalyst with a synergistic effect to promote all of the beneficial microbes in the environment, and these microorganisms secrete proteins, organic acids, and antioxidants in the presence of organic matter and convert them into energy, resulting in the transformation of a disease-inducing soil into a disease-suppressive soil [7]. Despite its widespread use in crop production, particularly in organic agriculture, the microbial profiles of PG during fermentation process have not been thoroughly investigated. Although the beneficial microbes present in the PG, their complete bacterial profiles are not yet understood due to limitations in culturing several genera/species using conventional microbiological techniques. Therefore, a culture independent technique (high-throughput sequencing analysis) was used in this study to better understand the microbial communities involved in PG production during fermentation period, while culture dependent techniques, on the other hand, are inherently inadequate to reveal greater microbial diversity. Furthermore, this study will also explore their potential agricultural applications.

Materials and Methods

Preparation of Panchagavya and Sample Collection

For the preparation of Panchagavya, fresh cow products were collected including Dung, Urine, Milk, Curd (fermented milk) and Ghee (clarified butter).

In addition, to improve the fermentation process, additional ingredients were added such as well ripened bananas, black organic jaggery, fresh tender coconut water and grape juice. All the ingredients were added in the proper ratio by following the protocol given by Tamil Nadu Agriculture University, India [16]. Followed by the addition of all ingredients, a soft white cloth was wrapped around the container to allow for proper aeration and to prevent other flies from dropping into the container. The contents were kept at room temperature for 15 days and it was necessary to mix the concoction every day with a clean stick in both a clockwise and an anticlockwise rotation. To carry out the study, samples were collected on the 7th day, 14th day, and 21st day respectively.

DNA Extraction and PCR Analysis

Before DNA extraction, the Panchagavya samples underwent thorough mixing through vigorous shaking to ensure content homogenization. Subsequently, total DNA was extracted from 5 ml of each collected sample using the Faecal/Soil Total DNA™ extraction kit (Zymo Research Corporation, CA, USA), following the manufacturer's instructions. The extracted DNA was then quantified using standard fluorometric techniques, and its purity was assessed based on the A260/280 and A260/230 ratios obtained from a Bio-drop spectrophotometer (Biochrom, Cambridge, UK). Following quantification and purity assessment, the DNA was preserved at -20ºC until further processing. The PCR and Sequencing methods were followed as described by Sibanda et al. [17]. Briefly, the initial PCR amplification of the whole variable region of 16S rRNA was amplified using the 27F and 1492R under following cycling conditions (95ºC, 5 min; 32 x [95ºC, 1 min; 55ºC, 1 min; 72ºC, 1 min]; 72ºC, 7 min; 4ºC, ∞). Followed by the second PCR amplification was carried out to cover the V1-V3 hypervariable region of 16S r RNA using 27F and 518R primer pairs, fused with Miseq adapters and heterogeneity spacers compatible with Illumina indexes for multiplex sequencing, as described by Selvarajan et al. [18]. Cleaning of the resultant PCR product, index library preparation, pooling and sequencing on Illumina Miseq to generate paired 300-bp high-quality reads of the V1-V3 region were performed as described by Selvarajan et al. [18]. Pooled libraries were subsequently sequenced on Illumina MiSeq platform with *v*3 chemistry (2x300 cycle's kit) (Illumina Inc., San Diego, CA, USA) at university of South Africa (UNISA), South Africa.

Statistical Analyses

Trimmed raw sequences (fastq files) were processed with the ngsShoRT (next-generation sequencing short reads) trimmer algorithm then merged and analysed with Mothur pipeline v.1.40.0 [19]. Sequence reads were quality filtered, chimeric sequences were removed using the UCHIME algorithm [20], and quality reads were classified using the Nave Bayesian classifier algorithm [21] against the SILVA database version 132 [22]. Clustering and assigning OTUs at the phylum, class, order, family, and genus levels was accomplished using a pairwise distance (Euclidean distance) matrix algorithm based on mothur's "*dist.seqs*" command with parameter "*cutoff* = 0.03" and the "cluster" command with the default "*furthest neighbor*" option [19]. Singletons and OTUs associated with mitochondria and chloroplasts were eliminated. At a genetic distance of 0.03, the alpha and beta diversities, including the Shannon-Weaver, Simpson, and Chao-1 indices, were calculated, and used to estimate within microbial communities.

Results

To characterize the total bacterial community structure, DNA was extracted from three samples collected at different day intervals and sequenced on a high throughput sequencing platform. Low-quality sequences in the raw fastq files were filtered out. After removing low-quality sequences and singletons, amplicon-based analysis of the V1-V3 of the bacterial 16S rRNA yielded 93,522 high-quality sequences ranging from 12,432 to 41,704 with an average read length of 527 bp. Good's coverage across the samples was >99%, indicating that the sampling depth was sufficient to estimate the microbial diversity encompassing all major bacterial groups inhabiting the Panchagavya samples. All three samples yielded a total of 5144 OTUs with an average of 1714 OTUs. When compared to both the Day 7 (375) and Day 21 (2311) samples, the number of OTUs that were identified in the Day 14 sample (2458) was significantly higher. This suggests that Day 7 apparently started with a reasonable degree of bacterial diversity. Subsequently, throughout the course of the fermentation process, other bacterial communities evolved and reached their peak on Day 14. There was a subsequent decline on Day 21, possibly as a result of increased microbial competition or a deficiency in nutrients. The Shannon-Weaver and Simpson indices showed differences in biodiversity among the different samples collected. The Shannon diversity magnitudes are as follows: Day 14 (4.52) >Day 21 (4.22)>Day 7 (1.71). Additionally, the different types of species are in a total community were identified by the Simpson indices for all samples, with Day 7 showing greater infinite diversity than the remaining samples of Day 14 and Day 21 respectively. The detailed demonstration of valid reads, OTUs, Shannon and Simpson diversity are given in Fig. 1a). This was further confirmed by Principal coordinate analysis (PCoA), in which the Day 14 and 21 samples are grouped together in terms of microbial diversity, while the Day 7 sample showed varying diversity compared with other samples (Fig. 1b).

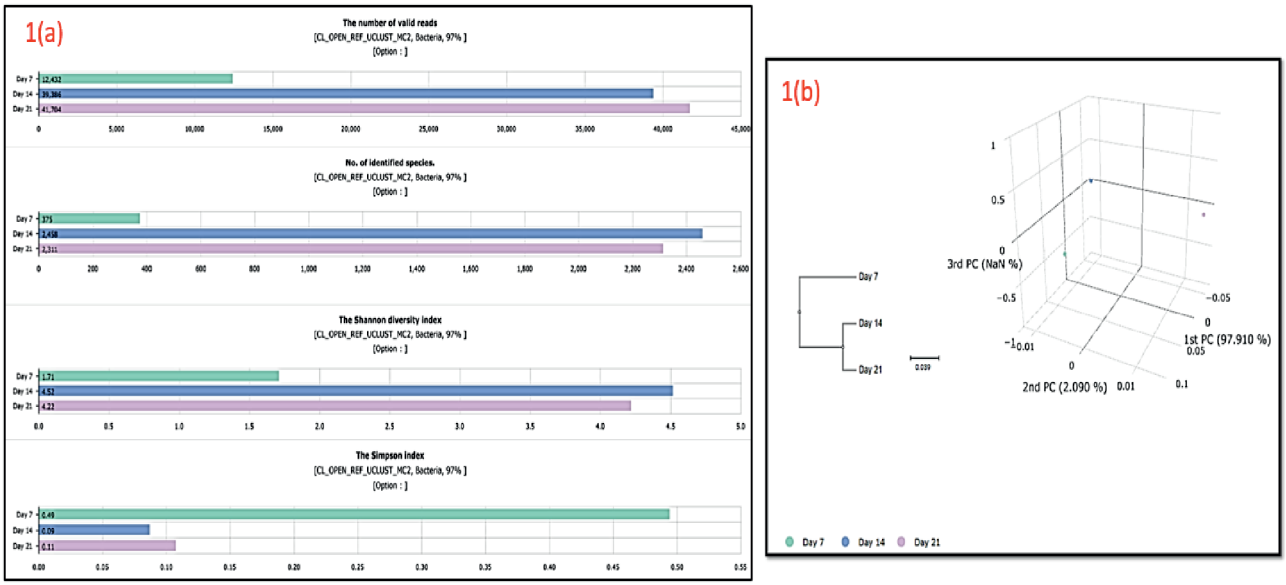

Fig. 1. Total Valid reads, Operational Taxonomic Units (OTUs), Shannon and Simpson diversities of the samples (1a); Principal coordinate analysis (PCoA) of bacterial diversity for the collected samples (1b).

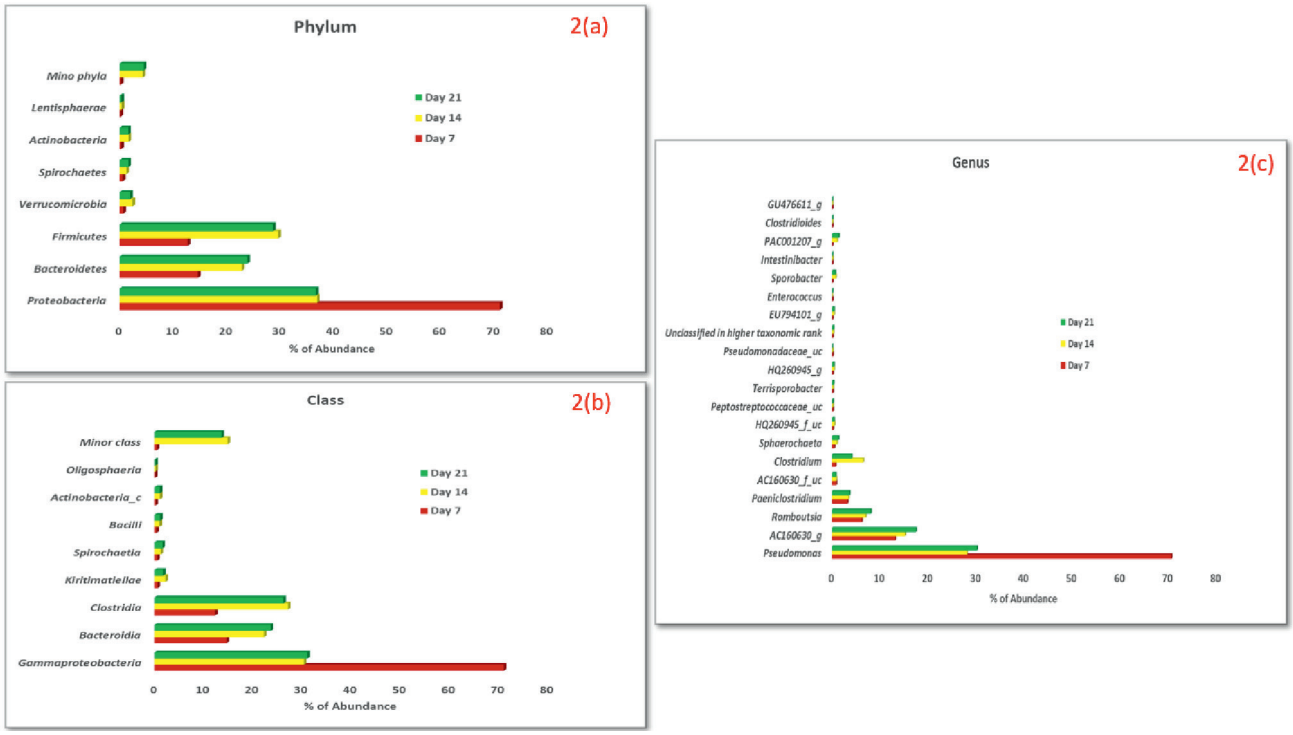

Fig. 2. Bacterial phylum level distribution (2a), class level distribution (2b) and Gens level distribution (2c) of the collected samples.

The study of the bacterial population revealed a total of 45 phyla, 92 classes, 168 orders, 333 families, 899 genera, and 2026 species. A wide range of phyla was present in the samples, but their relative abundances varied greatly (*Proteobacteria; Bacteroidetes; Firmicutes; Verrucomicrobia; Spirochaetes; Actinobacteria; Lentisphaerae*). *Proteobacteria* and *Bacteroidetes* were found in greater abundance at all samples, with *Proteobacteria* being the most common in all samples, followed by *Bacteroidetes* and *Firmicutes* (Fig. 2a). At the class level (Fig. 2b), the most common classes with discernible variations across the collected samples were *Gammaproteobacteria, Bacteroidia, Clostridia, Kiritimatiellae*, *Spirochaetia, Bacilli, Actinobacteria*, and *Oligosphaeria*. *Gammaproteobacteria, Bacteroidia*, and *Clostridia* were

found to be the most prevalent classes in all the samples studied. Other important bacterial classes that were found in the samples include *Epsilonproteobacteria, Betaproteobacteria, Dehalococcoidia, Deltaproteobacteria, Mollicutes, Lentisphaeria, Negativicutes, Tissierellia, Coriobacteriia, Paceibacter_c, Phycisphaerae, Synergistia, Alphaproteobacteria,* and *Verrucomicrobiae*.

Fig. 2c) clearly demonstrates the abundance of the different bacterial genera within each sample interval, providing distinctive evidence that the samples represent a wide variety of bacterial species. *Pseudomonas, AC160630, Romboutsia, Paeniclostridium, AC160630_f_uc, Clostridium, Sphaerochaeta, HQ260945_f_uc, Peptostreptococcaceae_uc, Terrisporobacter, HQ260945, Pseudomonadaceae, EU794101, Enterococcus, Sporobacter, Intestinibacter, PAC001207, Clostridioides* are the genera with the highest frequency of occurrence among the bacterial genera identified across all samples. The sample taken on day 7 had the highest concentrations of *Pseudomonas*; however, the abundance continued to gradually decrease through days 14 and 21. *Rombuoutsia, clostridium and Paeniclostridium* were the other major genera distributed in all the samples. Other notable genera include *Acidaminococcus, Acidibacter, Adiaphora, Acinetobacter, Actinoallomurus, Actinomadura, Actinomycetospora, Actinoplanes, Adlercreutzia, Advenella, Aequorivita, Aerococcus, Aeromonas, Aestuariicella, Cellulomonas, Cellulosibacter, Cellulosilyticum, Cellulosimicrobium, Cellvibrionaceae, Chitinispirillaceae, Chitinispirillum, Chitinophagaceae, Chryseobacterium, Citricoccus, Clavibacter, Lactobacillus, Lachnospira, Lactivibrio, Latescibacter, Legionellaceae, Leifsonia, Lentimicrobiaceae, Lentimicrobium, Leptolinea, Leuconostoc and Yersinia*. Important species such as *Pseudomonas caeni, Romboutsia timonensis, Paeniclostridium ghonii group, Romboutsia sedimentorum, Lactobacillus buchneri group, and Lactobacillus oligofermentans* group were found in all samples.

Day 7

Sample Day 7 exhibited 12, 432 valid reads having the average read length about 458 bp, including the 169 species. The total number of OTUs (375) are very low compared to the other samples. The major phylum includes *Proteobacteria* (70.98%), followed by *Bacteroidetes* (14.59%), *Firmicutes* (12.67%). Within the class *Gammaproteobacteria* (70.87%) was most abundant, followed by *Bacteroidia* (14.59%), *Clostridia* (12.26%) and *Kiritimatiellae* (0.62%) were observed. The major genus accounted in the Day 7 sample are *Pseudomonas* (70.64%), followed by *Rombuoutsia* (6.23%), *Paeniclostridium* (3.28%), *Clostridium* (0.76%) and *Sphaerochaeta* (0.5%). The main lactic acid bacterial members are *Lactobacillus paracasei* and *Lactobacillus reuteri*.

Day 14

Sample Day 14 exhibited 39, 386 valid reads having the average read length about 453 bp, including the 1390 species. The total number of OTUs (2458) are high compared to the other samples. The major phylum includes *Proteobacteria* (36.83%), followed by *Firmicutes* (29.58%), *Bacteroidetes* (22.79%), *Verrucomicrobia* (2.32%)*, Actinobacteria (1.63%) and Spirochetes* (1.24%). Within the class, *Gammaproteobacteria* (30.28%) was most abundant, followed by *Clostridia* (27.05%), *Bacteroidia* (22.18%), *Epsilonproteobacteria* (3.59%), *Kiritimatiellae* (2.19%), *Betaproteobacteria* (2.018%), *Spirocheatia c* (1.24%) and *Actinobacteri_c* (1.08%) was observed. The major genus accounted in the Day 14 sample are *Pseudomonas (28.11%),* followed by *Rombuoutsia* (7.05%), *Paeniclostridium* (3.48%), *Arcrobacter* (3.23%), *Sphaerchaeta* (1.6%), *Sporobacter* (0.8%). *Clostridium* (0.76%) and *Thiopseudomonas* (0.7%). The main lactic acid bacterial members are *Lactobacillus buchneri* and *Leuconostoc mesenteroides.*

Day 21

Sample Day 21 exhibited 41, 704 valid reads having the average read length about 453 bp, including the 1316 species. The total number of OTUs (2311) are high compared to the Day 7 and little low with Day 14 samples. The major phylum includes *Proteobacteria* (36.61%), followed by *Firmicutes* (28.62%), *Bacteroidetes* (23.81%), *Verrucomicrobia* (1.89%), *Actinobacteria* (1.58%) and *Spirochetes* (1.60%). Within the class, *Gammaproteobacteria* (31.01%) was most abundant, followed by *Clostridia* (26.12%), *Bacteroidia* (23.58%), *Epsilonbacteria* (3.79%), *Kiritimatiellae* (1.73%), *Betaproteobacteria* (0.9%), *Spirocheatia c (*1.60%) and *Actinobacteri_c* (1.06%) was observed. The major genus accounted in the Day 21 sample are *Pseudomonas* (30.17%), followed by *Rombuoutsia* (8.09%), *Clostridium* (4.10%) *Paeniclostridium* (3.67%), *Arcrobacter* (3.59%), *Sphaerchaeta* (1.36%), *Sporobacter* (0.71%) and *Trichococcus* (0.81%). The main lactic acid bacterial members are *Lactobacillus oligofermentans* and *Leuconostoc mesenteroides.*

Lactic Acid Bacteria and Gut Bacteria

Fig. 3 displays the main lactic acid producing bacteria (LAB) and gut bacterial members observed across all the samples. Among the LAB, the predominant members are *Bifidobacterium, Fructobacillus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus,* and *Weissella*. Notably, the genus *Lactobacillus* is the most abundant, comprising species such as *Lactobacillus oligofermentans, Lactobacillus buchneri, Lactobacillus paracasei, Lactobacillus suebicus,* and *Lactobacillus reuteri*. On the other hand, the predominant human gut bacterial members across all the samples include

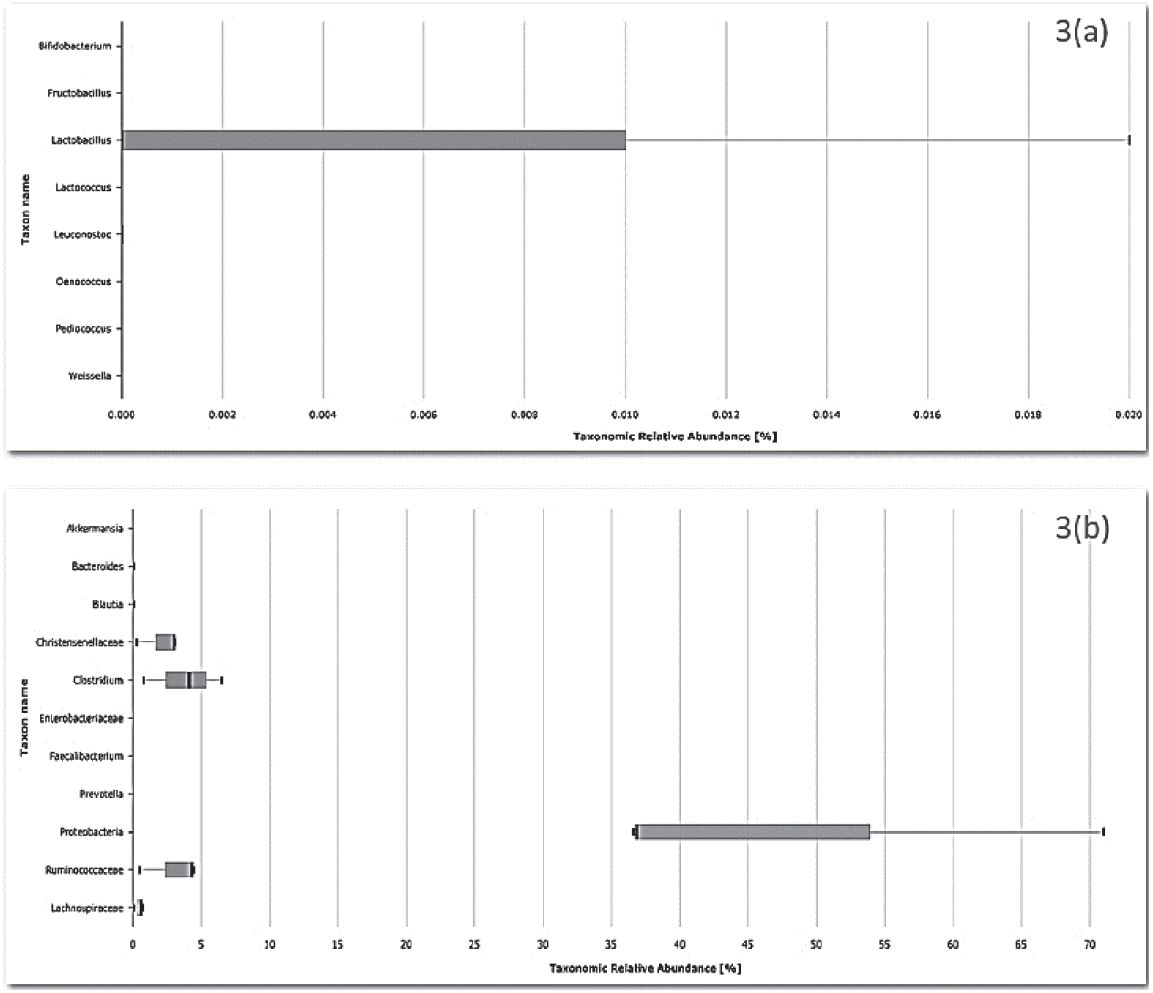

Fig. 3. Lactic Acid Bacterial (LAB) members (3a); Human Gut Bacterial members (3b) across all samples.

Clostridium, Christensenellaceae, Ruminococcaceae, Prevotella, Faecalibacterium, Enterobacterium, Blautia, and *Bacteroides.*

Discussion

Panchagavya is a liquid bio-manure made through fermentation of five ingredients from cows, namely, dung, urine, milk, curd, and clarified butter [23]. Despite significant evidence of PG's nutrient profile, fatty acid profile, proximate content, and phytohormone profile [5, 24], and a few studies on bacterial communities [1, 11], neither detailed investigation has yet been done to investigate the succession of bacterial diversity during the fermentation of PG. In this study, the bacterial community structure of Panchagavya was determined by sequencing 16S rRNA gene amplicons through an Illumina MiSeq approach. To best of our knowledge, this is the first attempt is being made to understand the succession of bacterial diversity in PG, which is a traditional Indian liquid organic plant growth promoter, using a high throughput sequencing analysis. Primers that targeted V3 regions have been utilized in the past for the purpose of species identification [25]. This study used primers that covered the V3-V4 regions of the 16s rDNA, and this region provided sufficient phylogenetic information about the bacteria in the samples [26], which is evidenced in the results of Goods coverage. Shannon diversity index is one of the most widely used parameters to assess biodiversity, and it measures the average degree of species distribution in each individual's population when it is drawn at random from a larger population. It is a mathematical measure of species diversity in a given community that is calculated based on the species richness (the number of species present) and the abundance (the number of individuals present) [27]. The present study showed the Shannon diversity magnitudes are as follows: Day 14 (4.52)>Day 21 (4.22)>Day 7 (1.71). Additionally, diverse bacterial communities were identified by the Simpson indices for all samples, with Day 7 showing greater infinite diversity than the remaining sampling sites of Day 14 and Day 21 respectively. However, the Shannon diversity displayed in another study from the 15th and 21st day samples was much lesser 2.96 and 2.82 respectively than this study [1], indicating the sequence depth of Illumina sequencing technology have been significantly

increased the resolution of diversity and function of microbial niches, which could not be addressed using conventional methods [28]. Consequently, the limitation of nutrient sources or competitiveness of bacterial must have caused a shift in Shannon diversity from 14th day to 21st day bacteria. A similar trend was seen in the earlier study, whose bacterial succession was noted during PG making using DGGE analysis [1].

Results of phylum revealed that *Proteobacteria* and *Bacteroidetes* were found in higher abundance at all sampling sites, with *Proteobacteria* being the most prevalent in all samples followed by the *Bacteroidetes* and *Firmicutes* respectively. Similarly, the abundance of *Proteobacteria* was reported in different cow products and Panchagavya [1, 11, 23]. The important genus identified across the samples are *Pseudomonas, Romboutsia, Paeniclostridium, Clostridium, Sphaerochaeta, HQ260945_f_uc, Peptostreptococcaceae_uc, Terrisporobacter, HQ260945_g, Pseudomonadaceae_uc, EU794101_g, Enterococcus, Sporobacter, Intestinibacter, PAC001207_g, Clostridioides*. Hence, this the first study to explore detailed bacterial communities, there are no early research to compare the main genera, however these genera are widely accepted members for their biotechnological potential and agricultural applications. Genus *Pseudomonas* was a dominant member exhibited throughout the fermentation process (Fig. 2c), it has been well reported that genera *Pseudomonas putida* and *Pseudomonas fluorescens* were actively associated with plant growth and involved in imparting induced systemic resistance (ISR) in the crop plants [29, 30]. In addition, extracellular lipases from *Pseudomonas* play an important role in biotechnological and industrial processes due to their application in biofuels, food, and pharmaceutical industries [30]. The *ACl60630* genus was recently reported to be present in anaerobic digesters to participate in the production of biogas [31], was found to be the second most prevalent genera in the PG. Despite their prevalence in the GIT and association with indole, skatole, isobutyric acid, isovaleric acid, and amino acid fermentation in the absence of carbohydrates, relatively little is known about the genus *ACl60630* [32]. *Romboutsia* species are flexible anaerobes that are adapted to a nutrient-rich environment in which carbohydrates and exogenous sources of amino acids and vitamins are abundantly available [33]. This bacterial genus succession was high during the fermentation process, and it was recently reported as a potential member for nitrogen and phosphorous recovery [34].

Next to the dominant bacterial members, in this study members of fermentative acidogenic bacteria (FAB), reported high (Fig. 2c). Zhao et al. [35] stated that FAB play a key role in breakdown of organic matter to yield hydrogen, ethanol, and volatile fatty acids (VFAs). FAB members such as *Clostridium* and *Pseudomonas* convert monomers to VFAs such as acetate, propionate, butyrate, isobutyrate, valerate and isovalerate, which are then utilized by SRB members as electron donors for efficient reduction processes [36]. Additionally, *Pseudomonas* also associate with FAB to degrade diverse chemical pollutants in soil [37]. Similarly, the human bacterial gut members were also identified in this study including *Clostridium, Christensenellaceae, Ruminococcaceae, Prevotella, Feaclibacterium, Enterobacterium, Blautia,* and *Bacteroides*. Notably, the member *Ruminococcus* an important fibrolytic bacterium of the rumen, can ferment hexoses and pentose's as well as cellulose and hemicelluloses to produce hydrogen from energy crops such as sweet sorghum [38]. Importantly, a carboxymethyl cellulase (CMCase) gene from *Prevotella* bacterial member was used to construct a shuttle vector for the production of different enzymes, thereby in future this members of bacteria play an important role in production of industrial important enzymes [39].

Apart from the gut bacterial members, the study also identified lactic acid bacterial members in the samples. The results demonstrated the presence of various bacterial genera, including *Bifidobacterium, Fructobacillus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus,* and *Weissella*. Among these, the genus *Lactobacillus* emerged as the predominant member, encompassing species such as *Lactobacillus oligofermentans*, *Lactobacillus buchneri, Lactobacillus paracasei, Lactobacillus suebicus,* and *Lactobacillus reuteri*. These bacteria have been found to play a direct role in activating cytokinin, a significant growth-promoting hormone [40]. Additionally, the genus Lactobacillus, belonging to the family Lactobacillaceae, is known for its ability to produce exopolysaccharides (EPS) and has been investigated for various biotechnological applications. Notably, certain Lactobacilli strains have been studied for their potential in reducing cholesterol levels. Among them, *L. reuteri* NCIMB 701089 exhibited promising potential in assimilating cholesterol in the intestine, suggesting its suitability for cardiovascular disease therapy [41]. Moreover, our study revealed a multitude of bacteria genera in the Panchagavya that have not been characterized so far for any role in the soil and plant system, urging for a comprehensive investigation into the potential functions of these bacteria.

Conclusion

To conclude, a high throughput sequencing technique was employed in attempting to unravel the succession of the bacterial community structure that occurs during the fermentation process of organic liquid manure. The succession of bacterial communities initially started on the 7th day of the fermentation process, showed significant improvement on the 14th day, and then started to decline on the 21st day. There are a few bacteria that have not yet been assigned a classification, indicating that it may contain bacteria with a wide variety of functions that have not yet been revealed. In addition,

the classified bacteria have a significant application potential in the agricultural and biotechnological fields, which can be further investigated with appropriate cultivation techniques. Therefore, Panchagavya is the best alternative to maintain sustainable agricultural production without influencing the natural ecosystem.

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Conflict of Interest

The authors declare no conflict of interest.

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