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Revisiting the oldest manure of India, *Kunapajala*: Assessment of its animal waste recycling potential as a source of plant biostimulant

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India's oldest documented manure, most commonly referred to as *Kunapajala*, has a long history of over 1,000 years in crop cultivation. *Kunapajala* is primarily an *in-situ* decomposition technology of animal waste and can potentially provide an eco-friendly pipeline for recycling bio-waste into essential plant nutrients. This traditional animal manure, in addition, also contains dairy excreta (e.g., feces and urine), dairy products (e.g., milk and ghee), natural resources (e.g., honey), broken seeds or grains, and their non-edible by-product waste. Here, we aimed to assess the waste recycling and plant biostimulant potential of *Kunapajala* prepared from livestock (e.g., Black Bengal goats) or fish (e.g., Bombay duck) post-processed wastes over different decomposition periods, e.g., (0, 30, 60, and 90-days). In this study, an *in-situ* quantification of livestock- (*IKPJ*) and fish-based *Kunapajala* (*fKPJ*) reveals a dynamic landscape of essential plant primary nutrients, e.g., $(0.70 > \text{NH}_4\text{-N} < 3.40 \text{ g}\cdot\text{L}^{-1})$, $(100.00 > \text{P}_2\text{O}_5 < 620.00 \text{ mg}\cdot\text{L}^{-1})$, and $(175.00 > \text{K}_2\text{O} < 340.00 \text{ mg}\cdot\text{L}^{-1})$, including other physico-chemical attributes of *Kunapajala*. Using correlation statistics, we find that the plant-available nutrient content of *Kunapajala* depicts a significant ($p < 0.0001$)

transformation over decomposition along with microbial dynamics, abundance, and diversities, delineating a microbial interface to animal waste decomposition and plant growth promotion. Importantly, this study also reports the indole 3-acetic acid (IAA) content ($40.00 > \text{IAA} < 135.00 \text{ mg}\cdot\text{L}^{-1}$) in *Kunapajala*. Furthermore, the bacterial screening based on plant growth-promoting traits and their functional analyses elucidate the mechanism of the plant biostimulant potential of *Kunapajala*. This assay finally reports two best-performing plant growth-promoting bacteria (e.g., *Pseudomonas chlororaphis* and *Bacillus subtilis*) by the 16S ribotyping method. In support, *in-planta* experiments have demonstrated, in detail, the bio-stimulative effects of *Kunapajala*, including these two bacterial isolates alone or in combination, on seed germination, root-shoot length, and other important agronomic, physio-biochemical traits in rice. Together, our findings establish that *Kunapajala* can be recommended as a source of plant biostimulant to improve crop quality traits in rice. Overall, this work highlights *Kunapajala*, for the first time, as a promising low-cost microbial technology that can serve a dual function of animal waste recycling and plant nutrient recovery to promote sustainable intensification in agroecosystems.

KEYWORDS

Kunapajala, animal waste, waste recycling, nutrient recovery, plant growth regulators (PGRs), plant growth-promoting bacteria (PGPB)

1. Introduction

To meet the increasing global demands for better quality food, intensified crop production in combination with large-scale livestock farming has been contributing to on-farm residue generation, including animal waste, at an astonishingly rapid growth rate in the modern era. India, as an example, produces about 683 million tones of crop residues per year of 10 major Indian crops (Bhattacharjya et al., 2019), while rice and wheat alone contributed around 300 million tones of residue generation recorded in the year 2017–2018 (Venkatramanan et al., 2021). The data on livestock, on the other hand, estimates ~135 million tones of animal excreta generation per year in India, of which more than 90% are from cattle and buffalo sources (Bhattacharjya et al., 2019). In addition, India also accounts for 11% of goat meat and nearly 8% of fish production, ranking as the world's second and third largest producers, respectively (Norris and Smith, 2020). According to the 20th Livestock census data, livestock industries, including fisheries, contribute more than 25% of the total agriculture GDP in India, with an overall increase of 4.6% in production in the last 7 years. The post-processed by-products generated daily in these growing livestock and fish industries in India and the rest of the world, in turn, have led to a gigantic bio-waste accumulation of serious environmental concerns and seek immediate attention for careful management. Recent studies evaluated and discussed the scope of available biomass

residues, including leftover agricultural residues and animal and municipal waste, in bioenergy production in India (Singh et al., 2022). In addition, biomass conversion into manures and compost can be an alternate mode of bio-waste management with a possible application in agriculture. Several studies confirmed that bio-waste, including crop residues and animal excreta, are potential sources of essential plant nutrients (e.g., N, P, K) and plant biostimulants (e.g., plant growth-promoting microbes, phytohormones, protein hydrolysates) and can be suitably recycled back into the agroecosystems (Huang et al., 2021). Therefore, the technological services in agriculture and allied sectors have become increasingly instrumental in transforming the circular economy associated with bio-waste management in recent times (Paes et al., 2019; Bakan et al., 2022). At present, advanced technologies, such as anaerobic digestion (AD), composting, algal-based sewage treatment and resource recovery systems (STARR), are commercially available to promote the recycling of “waste-turn-into-wealth” and are reported to have tremendous potential to minimize the menace of waste hazards, posing environmental and public health risks (Onwosi et al., 2017; Abeyisiriwardana-Arachchige et al., 2021; Cremonez et al., 2021). On a similar goal, the technologies that recycle crop residues, animal wastes, and human excreta can provide alternate options to convert bio-degradable solid waste into agricultural inputs in a sustainable way (Ahuja et al., 2020; Kelova et al., 2021; Greff et al., 2022). The main aim of these technologies, in general, is to maximize nutrient recovery and

the fertilizer potential of derived by-products. In addition, there are also reports of indirect application-based robust methods for safe recycling in domestic sewage treatment, municipal, and agro-industrial bio-waste for further use in agriculture (Gross et al., 2021; Lin et al., 2021; Ravindran et al., 2021). These technologies, in summary, promote the waste recycling process, create an alternate nutrient cycle, and maintain soil health in agricultural lands, and as a result, can significantly reduce the adverse loads of synthetic fertilizers and pesticides in the agroecosystems.

In India, several traditional formulations have been documented for their application in crop farming for over a 1,000 years. *Kunapajala* (a Sanskrit word meaning filthy fluid) is an ancient innovation of animal waste recycling into agricultural inputs. This liquid animal manure, narrated originally in “*Vrikshayurveda*” by Surapala around 1,000 AD, is a formulation of decomposed animal waste such as bones, viscera, fins, and scales from fish waste or waste of crushed bones, skins, and flesh derived from livestock including cattle, goats, pigs, and sheep (Nene, 2018). In addition, locally available resources such as dairy excreta (e.g., cow dung and cow urine), broken or damaged pulse seeds (e.g., green gram or black gram), crop residues, or non-edible by-products obtained after oil extraction (e.g., rice husks and oil cakes), natural or forest resources (e.g., honey), dairy products (e.g., milk and ghee) are also used either as raw or processed materials and most commonly considered as enrichment agents or bulking materials in the *Kunapajala* preparation (Nene, 2012). Based on end-product trend analyses of animal waste decomposition (Ahuja et al., 2020; Brod and Øgaard, 2021), this low-cost animal manure is assumed to provide a rich source of plant nutrients, the majority of which are in the form of N, P, and K (Chakraborty et al., 2019). Hence, this technology of great potential can serve a dual purpose of sustainable animal waste management and an alternate mode of nutrient cycling in agriculture (Sorathiya et al., 2014). On the other hand, manure composition is dynamic and varies with changing farming circumstances and higher eco-social requirements (e.g., conventional vs. organic farming) (He et al., 2016). Thus, updated knowledge of the oldest manure of India is needed to optimize its recycling potential.

Kunapajala is also an abundant source of plant growth-promoting bacteria (PGPB) that could offer various benefits to its host plants, including nutrient availability, plant growth promotion, and control of pests and diseases (Chakraborty et al., 2019). Therefore, it has been recommended widely as a foliar spray or soil drenching for several crops, such as rice (*Oryza sativa*), mustard (*Brassica campestris*), and black gram (*Vigna mungo*), however with more emphasis on vegetables in India, such as okra (*Abelmoschus esculentus*), tomato (*Solanum lycopersicum*), chili (*Capsicum annuum*), and cowpea (*Vigna unguiculata*) (Mishra, 2007; Ali et al., 2012; Deshmukh et al., 2012; Sarkar et al., 2014; Kavya and Ushakumari, 2020). In addition, the *Kunapajala* formulation also showed a significant

impact on the growth, physiological, biochemical, yield, and quality attributes of medicinal plants, *Ashwagandha* (*Withania somnifera*), and *Kalamegha* (*Andrographis paniculata*) (Ankad et al., 2017, 2018). However, despite in-depth physico-chemical and microbiological characterization of *Kunapajala* (Jani et al., 2017; Chakraborty et al., 2019) and its positive effect on plant growth, no attempt has been made, to date, to understand the population dynamics of microbes in *Kunapajala* and their functions in animal waste decomposition, nutrient recycling, and mineralization processes. In addition, functional screening of the PGPB isolates, owing to their combinatorial, bio-stimulative impact on the *Kunapajala* formulation in relation to plant growth and development, remains largely unexplored.

The present study describes the trend analyses of livestock- (IKPJ) and fish-based *Kunapajala* (fKPJ) throughout decomposition (e.g., 0, 30, 60, and 90-days) to maximize the recovery of resources in terms of available plant nutrients, plant growth regulator content, and as a source of the microbial niche contributing to plant growth-promoting traits such as IAA-production, N-fixation, P- and K-solubilization. We then screened bacteria for their efficiency in plant growth promotion and reported the best-performing isolates using the standard 16S ribotyping method. Finally, this study also assessed the plant biostimulant potential of *Kunapajala*, including these two bacterial isolates, on different growth stages of rice in a pot-based assay.

2. Materials and methods

2.1. Livestock and fish waste-derived *Kunapajala* preparation

In order to assess and compare various quality attributes, the preparation of *Kunapajala* and its components used in this study has been adopted based on the recommendation of Nene, a modified version of the ancient formulation (Jani et al., 2017; Nene, 2018). Here, the *Kunapajala* samples were prepared separately from two different sources of waste: livestock waste (e.g., Black Bengal goats: *Capra hircus*) collected from the slaughterhouse; and fish waste (e.g., Bombay duck: *Harpadon nehereus*) from a local fish market. Subsequently, these wastes were boiled and mixed with other ingredients to prepare the *Kunapajala* formulation (see Supplementary Table A1 for details). Briefly, livestock and fish waste (having a fresh weight of about 1.25 kg) with crushed bones, fins, skins, and marrows were boiled in 2.5 L of water at 100°C for an hour in two separate containers. After cooling down, the liquid residue was added to 5.0 L of water along with rice husks, available oil cakes (e.g., Mustard: *Brassica campestris*), and broken or damaged pulse seeds (e.g., Green gram: *Vigna radiata*). The other ingredients, such as cow dung, cow urine, cow milk, ghee, and honey, were then serially added in the amounts

specified to these preparations and were adjusted with water to 50.0 L. The mixture was then kept in the container under shade at room temperature (25–30°C), stirred twice daily during the incubation, and the mouth of the container was further closed with a cloth to facilitate aeration, the prevention of houseflies from laying eggs, and uniform decomposition of the components. Finally, the liquid manures were collected with the help of a fine net and diluted to 100.0 L to prepare the *Kunapajala* formulation. In this study, the samples were collected for analysis at 30-day intervals, starting from 0-day to 90-days of incubation, e.g., (0, 30, 60, and 90-days).

2.2. The pH, electrical conductivity, organic C, total soluble protein, and macro-nutrient concentrations in the *Kunapajala* manure

The physico-chemical attributes of the *Kunapajala* preparation were determined by following the standard methods. In brief, after mixing with deionized water at a 1:100 (v/v) ratio, the pH and the electrical conductivity (EC) of the *Kunapajala* samples were measured using pH meter (Systronics Digital) and conductivity electrode, respectively. It is important to note that the deionized water has poor conductivity, typically in the range of 10^{-5} – 10^{-6} $\text{mS}\cdot\text{cm}^{-1}$, and is therefore assumed to have no influence on the EC measurement. The wet oxidation (Walkley and Black, 1934) and the Lowry (Waterborg, 2009) methods were used to estimate oxidizable organic C and total soluble protein (TSP) content, respectively.

The available N ($\text{NH}_4\text{-N}$) of *Kunapajala* was then determined using the standard alkaline-based Kjeldahl technique (Subbiah and Asija, 1956). Similarly, available P (P_2O_5) and K (K_2O) were determined by Olsen et al.'s (1954) and by the flame photometric (Jackson, 1973) methods, respectively.

2.3. The diversity analysis of the microbial community

To determine the microbial population, *Kunapajala* was serially diluted at the desired concentration and plated with three replications per Petri plate on appropriate culture media so that microbes grow as distinct colonies. Then, the colonies were counted according to standard microbiological norms to study and calculate the microbial dynamics (Mukherjee et al., 2022). The specific growth media for a diverse group of microbes, including their standard cultural conditions, are further enumerated in Supplementary Table A2. The bacteria that appeared as single colonies on specific growth media were

streaked further to ensure the purity of cultures for various plant beneficial trait assays.

2.4. The efficacy assay of free-living N-fixers and P-solubilizing bacteria

Free-living N-fixers (FNFs) and P-solubilizing bacteria (PSB), thus obtained as pure colonies on culture plates, were cultured again in nutrient broth to study their plant beneficial traits. Now, to assess the ability of FNFs to fix atmospheric N, the bacteria were grown on a Luria-Bertani culture medium overnight, and then the freshly grown culture was inoculated again to Jansen's media. Finally, 8 mL of grown culture was collected after 7-days of incubation at 30°C for acid digestion to estimate the total N content by the Kjeldahl method (Subbiah and Asija, 1956). On the other hand, to determine the efficiency of PSB in solubilizing insoluble P sources, the diameter of the P-solubilization zone was taken as an indicative measurement during their growth on Pikovskaya's agar media at 30°C (Pikovskaya, 1948; Mukherjee et al., 2022). Furthermore, the efficiency of P-solubilization was again validated quantitatively by Olsen's extraction method (Olsen et al., 1954).

The best-performing FNF and PSB among these two classes of beneficial bacteria obtained in this screening were scored and compared based on their highest N-fixing and P-solubilizing attributes, respectively, and characterized further by subsequent 16S ribotyping and *in-planta* experiments.

2.5. Estimation of IAA production

The IAA content of the *Kunapajala* formulation was detected and quantified by the high-performance thin-layer chromatography (HPTLC) method (Goswami et al., 2015). Briefly, the *Kunapajala* extractants were first isolated using the ethyl acetate solvent. Then, the extractants were loaded on the TLC plate and air-dried. The spots thus formed on the TLC plate were scanned using Scanner 3 (Camag) in absorbance-reflectance mode at a 256 nm wavelength. In the case of the plant beneficial microbes, the bacterial isolates were grown on Luria-Bertani culture media with $0.10 \text{ g}\cdot\text{L}^{-1}$ of L-tryptophan. Then, 10 mL of grown culture was collected after 6- and 9-days of incubation to determine the total indole content by the standard Salkowski method (Salkowski, 1885).

2.6. Molecular identification of plant beneficial bacteria

Genomic DNA was extracted from two best-performing plant beneficial bacteria by the lysozyme-mediated lysis method (Moore et al., 2004) with a minor modification. This

protocol, however, did not include a preheated NaCl/CTAB solution. Then, using the primers [Forward primer (27F): 5'AGAGTTTGATYMTGGCTCAG3'; Reverse primer (1492R): 5'TACCTTGTTAYGACTT3'], PCR was performed to amplify the 16S rDNA region of the selected bacteria (Edwards et al., 1989). Next, the PCR-amplified products were gel purified according to the manufacturer's protocol (QIAGEN). The purified rDNA fragments thus obtained were sequenced by the Sanger di-deoxy method, and the generated nucleotide sequences were further run to search for homology by the NCBI Nucleotide BLAST program (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

2.7. A plant-based bioassay of *Kunapajala* and derived bacterial isolates of plant beneficial traits

Seeds of rice (*Oryza sativa*) from the Shatabdi (IET4786) variety were used in this experiment. Notably, Shatabdi is a popular, high-yielding rice variety cultivated in different agro-climatic zones of West Bengal, India. Studies on germination percentage and root-shoot length were executed by employing a plant-based assay based on the *Kunapajala* formulation and derived bacterial isolates. In this experiment, the selected seeds were first subjected to surface sterilization by stepwise washing with 70% ethanol and 0.5% hydrogen peroxide solutions for 3 min each, followed by 5 rinses in sterile distilled water. Next, to determine the effect of *Kunapajala* on germination and other agronomic parameters, sterilized seeds were further soaked and treated with seed priming agents for 6 h. The seed priming agents used in this study were 1% *IKPJ* and *fKPJ* formulations after different days of decomposition (e.g., 0, 30, 60, and 90-days), best FNF, and PSB isolated from the *Kunapajala* preparation, and sterile distilled water as a control. In the case of the best FNF and PSB, a fixed number of bacterial cells was counted ($\sim 3 \times 10^{10}$ cells in 30 mL of sterilized distilled water) and used to treat the seeds. It is important to note that the experiment comprises five replications with 100 seeds per replication. The study on germination percentage was performed on Petri plates under 10 h of light and 14 h of darkness for 7 consecutive days at 25–30°C. The emergence of root-shoot length, including the germination percentage, was carefully observed, recorded, and measured during this period.

The plant growth-promoting abilities of *Kunapajala* and derived bacterial isolates were evaluated on transplanted rice seedlings in a pot-based experiment. In this experiment, three healthy rice seedlings with a root length of about 3 mm were selected randomly from Petri plates and transplanted further in each earthen pot filled with 2.5 kg of air-dried and autoclaved soil. The details of the nutrient content and physico-chemical and microbiological properties of the agricultural soils used in

this study are available in [Supplementary Table A3](#). In this study, we followed a foliar spray of the *Kunapajala* formulation (e.g., @1% v/v) every 15-day interval up to the flowering stage of rice while considering the plant nutrient content and an earlier recommendation of *Kunapajala* (Mishra, 2007).

2.8. Agronomic and physico-biochemical attributes of rice seedlings

Shoot height, root length, fresh weight, and dry weight of shoots and roots of a tiller were recorded at every 15-day interval starting from 45-days after transplanting to till harvesting. In addition, other standard agronomic attributes such as the number of tillers per plant, the number of active tillers per plant, the number of grains per panicle, the chaffy: filled grain ratio, the 1,000 seed weight, and yield per plant after harvest were also examined and recorded.

Further, the content of the photosynthetic pigments, such as chlorophyll-*a*, chlorophyll-*b*, and total chlorophyll content, was estimated using the standard spectrophotometric method. Briefly, 50 mg of freshly chopped leaves were dipped in 80% acetone in dark conditions for 72 h at 10°C, followed by their intensity measurement at 645 and 663 nm wavelengths. All the quantitative calculations to estimate chlorophyll content follow the standard formula (Tao et al., 2022).

On the other hand, to determine the total carbohydrate and soluble protein content of different plant parts, roots, shoots, and leaves were collected and finely chopped into smaller pieces. Then, 50 mg of finely chopped plant parts were further acid-hydrolyzed to estimate the carbohydrate content by the standard colorimetric method (Jain et al., 2017). In the case of total protein content estimation, the same amount of plant parts was dissolved in phosphate-buffered saline (PBS) and further analyzed by following the Lowry method (Lowry et al., 1951).

2.9. Statistical analysis

Samples collected after different incubation periods were analyzed, with $n = 3$ in each case unless otherwise specified in the *in-planta* experiment (i.e., $n = 5$). The study used Dunnett's tests as a multiple comparison procedure for a significant One-way ANOVA. When the data breached test assumptions (e.g., non-normal data), we employed the non-parametric Kruskal-Wallis test as an alternative to One-way ANOVA. In that case, pairwise comparisons using Dunn's test were used to compare statistical differences among treatments. Significance values were further adjusted by the Bonferroni correction for multiple tests. Analyses were performed in SPSS for Windows 25.0 (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Similarly, Pearson's correlation coefficient analysis was

performed by a computer-based STAR program (<http://bbi.irri.org/products>).

3. Results

3.1. Biomass-degrading bacteria: An implication in the *Kunapajala* technology to recycle animal waste

To explore the microbial action of livestock and fish waste degradation in *Kunapajala*, we initially studied the microbial dynamics of proteolytic, lipolytic, and starch-hydrolyzing bacteria, henceforth named biomass-degrading bacteria, based on their colony-forming unit (CFU) on appropriate nutrient media (Supplementary Table A2). The idea to examine the dynamics of the starch-hydrolyzing bacterial isolates is due to the crop residues, including rice husks and cow dung, which are present in the *Kunapajala* formulation. In this study, we observed an elevated level of the biomass-degrading bacterial population (Table 1). However, till 90-days of incubation, there was no reduction observed in the microbial population specifically for this class of bacteria, except for the starch-hydrolyzing bacteria, which showed a decline in the population (Table 1). Overall, our data reveal that the trend in the population dynamics of the biomass-degrading bacteria is amazingly similar in *IKPJ* and *fKPJ*.

To monitor the decomposition process and depolymerization of organic matter subsequently, organic C and TSP were estimated at every 30-day interval till 90-days of incubation. The result indicates that the level of organic C is gradually depleted significantly ($p < 0.0001$) over time, at least up to 60-days, in both *IKPJ* and *fKPJ* (Table 1). It is important to note that the initial concentration of the organic C (in percent value) in two different *Kunapajala* formulations is in nearly identical ranges (*IKPJ*: $1.95 \pm 0.01\%$, and *fKPJ*: $1.79 \pm 0.03\%$). Similarly, the initial TSP content ($\text{g}\cdot\text{L}^{-1}$) of the *IKPJ* and *fKPJ* formulations ranges between 3.58 ± 0.02 and 3.98 ± 0.14 , respectively, and it also shows a similar trend of gradual depletion over a period of incubation. The correlation statistics further establish a positive interaction between microbial growth and animal waste decomposition in the *Kunapajala* technology (Supplementary Table A4). Together, these data indicate a possible role of microbial action as evident in the abundance of biomass-degrading bacteria in the initial steps of animal waste degradation in *Kunapajala*.

Besides, other factors such as pH, EC, moisture content, aeration, and temperature are critical in regulating microbial activity and thus may determine the waste decomposition rate in the *Kunapajala* system (Rastogi et al., 2020). The

study revealed that the pH in both of these formulations increased significantly ($p < 0.0001$) from the initial acidic pH (*IKPJ*: 4.70 ± 0.01 , and *fKPJ*: 4.47 ± 0.02) to nearly neutral (*IKPJ*: 7.17 ± 0.06 , and *fKPJ*: 6.87 ± 0.06) over decomposition (Table 1). The EC data ($\text{mS}\cdot\text{cm}^{-1}$) in *IKPJ* and *fKPJ*, on the other hand, range from 0.31 ± 0.003 to 1.21 ± 0.03 and 0.36 ± 0.01 to 1.29 ± 0.01 , respectively, and show no adverse fluctuation to a higher value over incubation (Table 1). Based on physico-chemical and microbiological analyses of *Kunapajala*, this study highlights for the first time a comprehensive nutrient-microbe network and further reports an amazingly uniform decomposition pattern in *Kunapajala*, irrespective of animal waste source and heterogeneity.

3.2. *Kunapajala* is the source of essential plant nutrients: An *in-situ* resource recycling mode to promote the nitrogen, phosphorus, and potash cycles in agroecosystems

In this study, we further assessed the bio-fertilizer potential of *Kunapajala* in terms of its plant nutrient concentration. An earlier report indicates *Kunapajala* formulation as a rich source of plant nutrients based on 40-days of decomposition (Chakraborty et al., 2019). Here, we have extended the earlier observation and evaluated, in particular, the plant nutrient content (e.g., available N, P, and K) of *Kunapajala* at every 30-day sample till 90-days of incubation. Our data indicate that the concentration of $\text{NH}_4\text{-N}$ and P_2O_5 increases significantly ($p < 0.0001$) at 60-days of waste decomposition in both the *IKPJ* and the *fKPJ* (Table 1). In comparison over different incubation periods, our study established that the overall concentration of $\text{NH}_4\text{-N}$, P_2O_5 , and K_2O reaches its optimum range at 60-days of incubation in *IKPJ* and *fKPJ* (Table 1). These data, in fact, correlate with the uniform animal waste decomposition, including the population dynamics of biomass-degrading bacteria (Supplementary Table A4). These results support a previous report (Chakraborty et al., 2019) that the *Kunapajala* manure is a rich source of plant nutrients, especially as a plant-available N, P, K ($0.70 > \text{NH}_4\text{-N} < 3.40 \text{ g}\cdot\text{L}^{-1}$; $100.00 > \text{P}_2\text{O}_5 < 620.00 \text{ mg}\cdot\text{L}^{-1}$; and $175.00 > \text{K}_2\text{O} < 340.00 \text{ mg}\cdot\text{L}^{-1}$). In summary, this study presents *Kunapajala* as a natural resource restoration technology that may offer enormous potential to provide an alternate source of plant primary nutrients in modern-day agriculture, considering an incremental mining operation followed by the gradual exhaustion of natural resource-based rock phosphate and potash mines and their limited availability in recent years.

TABLE 1 The microbiological and physico-chemical properties of *Kunapajala* after different days of incubation.

Treatment	IKPJ				fKPJ			
	0-day	30-days	60-days	90-days	0-day	30-days	60-days	90-days
I. Microbiological parameters								
Total bacteria ($\times 10^{10}$ CFU \bullet mL $^{-1}$)	3.33 \pm 0.09a	2.77 \pm 0.15b	3.27 \pm 0.19a	2.80 \pm 0.17b	0.80 \pm 0.21c	0.93 \pm 0.09c	0.33 \pm 0.13d	0.33 \pm 0.03d
Total proteolytic bacteria ($\times 10^9$ CFU \bullet mL $^{-1}$)	0.45 \pm 0.01h	0.69 \pm 0.01f	0.79 \pm 0.01d	0.84 \pm 0.01c	0.52 \pm 0.01g	0.72 \pm 0.01e	0.91 \pm 0.01b	0.94 \pm 0.003a
Total lipolytic bacteria ($\times 10^8$ CFU \bullet mL $^{-1}$)	0.22 \pm 0.01e	0.66 \pm 0.003b	0.77 \pm 0.01a	0.76 \pm 0.01a	0.14 \pm 0.01f	0.54 \pm 0.01d	0.66 \pm 0.01b	0.61 \pm 0.01c
Total starch-hydrolyzing bacteria ($\times 10^9$ CFU \bullet mL $^{-1}$)	0.10 \pm 0.01f	0.47 \pm 0.02c	0.60 \pm 0.02a	0.29 \pm 0.01e	0.12 \pm 0.01f	0.36 \pm 0.01d	0.55 \pm 0.01b	0.40 \pm 0.003d
Total FNFs ($\times 10^8$ CFU \bullet mL $^{-1}$)	1.00 \pm 0.12e	2.23 \pm 0.09b	2.93 \pm 0.12a	3.20 \pm 0.001a	0.23 \pm 0.07g	0.67 \pm 0.09f	1.70 \pm 0.17c	1.37 \pm 0.09d
Total <i>Rhizobium</i> bacteria ($\times 10^6$ CFU \bullet mL $^{-1}$)	2.67 \pm 0.67cd	8.33 \pm 1.45b	12.33 \pm 0.89a	3.00 \pm 0.58cd	1.33 \pm 0.33d	2.33 \pm 0.33cd	4.00 \pm 0.58c	4.67 \pm 0.33c
Total PSB ($\times 10^8$ CFU \bullet mL $^{-1}$)	0.70 \pm 0.06cd	0.97 \pm 0.03bcd	1.30 \pm 0.12b	1.37 \pm 0.03b	0.60 \pm 0.12d	1.13 \pm 0.09bc	2.03 \pm 0.09a	2.40 \pm 0.31a
Total KSB ($\times 10^5$ CFU \bullet mL $^{-1}$)	0.00 \pm 0.00d	0.37 \pm 0.03bc	3.43 \pm 0.18a	0.43 \pm 0.03b	0.00 \pm 0.00d	0.33 \pm 0.03bc	0.43 \pm 0.03b	0.17 \pm 0.03cd
Total <i>Pseudomonas</i> bacteria ($\times 10^9$ CFU \bullet mL $^{-1}$)	0.30 \pm 0.06d	0.60 \pm 0.06c	3.07 \pm 0.18b	3.40 \pm 0.12a	0.08 \pm 0.003d	0.16 \pm 0.01d	0.19 \pm 0.003d	0.24 \pm 0.02d
Total actinomycetes ($\times 10^{10}$ CFU \bullet mL $^{-1}$)	0.46 \pm 0.01b	0.16 \pm 0.02c	0.08 \pm 0.01de	0.13 \pm 0.01cd	0.53 \pm 0.05a	0.16 \pm 0.02c	0.07 \pm 0.003de	0.05 \pm 0.01e
Total fungi ($\times 10^6$ CFU \bullet mL $^{-1}$)	2.20 \pm 0.12b	4.27 \pm 0.09a	0.60 \pm 0.06de	0.27 \pm 0.03e	1.23 \pm 0.07c	1.37 \pm 0.07c	1.47 \pm 0.20c	0.70 \pm 0.15d
II. Physico-chemical parameters								
Organic C (%)	1.95 \pm 0.01a	1.81 \pm 0.02b	1.72 \pm 0.01c	1.68 \pm 0.003c	1.79 \pm 0.03b	1.71 \pm 0.01c	1.68 \pm 0.02c	1.61 \pm 0.03d
TSP (g \bullet L $^{-1}$)	3.58 \pm 0.02b	3.00 \pm 0.09cd	2.74 \pm 0.02d	2.38 \pm 0.03e	3.98 \pm 0.14a	3.47 \pm 0.11b	3.03 \pm 0.08c	2.96 \pm 0.06cd
pH	4.70 \pm 0.01f	5.79 \pm 0.01e	7.01 \pm 0.02b	7.17 \pm 0.06a	4.47 \pm 0.02g	5.96 \pm 0.02d	7.25 \pm 0.003a	6.87 \pm 0.06c
EC (mS \bullet cm $^{-1}$)	0.31 \pm 0.003f	0.42 \pm 0.02d	1.21 \pm 0.03b	0.42 \pm 0.003d	0.41 \pm 0.001d	0.46 \pm 0.01c	1.29 \pm 0.01a	0.36 \pm 0.01e
Available N (g \bullet L $^{-1}$)	0.88 \pm 0.07e	2.35 \pm 0.05d	2.69 \pm 0.05c	2.91 \pm 0.04b	0.77 \pm 0.05e	2.58 \pm 0.04c	2.73 \pm 0.05c	3.25 \pm 0.07a
Available P (mg \bullet L $^{-1}$)	247.24 \pm 14.93e	500.99 \pm 14.55b	613.88 \pm 2.53a	489.62 \pm 22.81b	109.63 \pm 5.18f	263.60 \pm 8.52e	367.00 \pm 8.31d	414.93 \pm 12.83c
Available K (mg \bullet L $^{-1}$)	265.44 \pm 1.80c	219.90 \pm 1.15d	222.50 \pm 1.43d	190.08 \pm 1.65e	329.77 \pm 3.16a	265.16 \pm 1.12c	319.69 \pm 0.44b	178.74 \pm 1.02f

Duncan's multiple range test was used to analyze the data, which are represented as the mean \pm standard error of samples ($n = 3$), followed by different letters indicating that the difference was statistically significant ($p < 0.05$). The results with the same letters were not significantly different ($p > 0.05$).

3.3. Screening and performance of plant beneficial bacteria isolated from the *Kunapajala* formulation

We next sought to study the growth dynamics of PGPB, such as FNFs and symbiotic N-fixers (e.g., *Rhizobium* species), along with PSB and K-solubilizing bacteria (KSB). We observed that the PGPB population ranges between 10^7 and 10^8 CFU•mL⁻¹ in the *Kunapajala* formulation, except for the KSB (Table 1). The population density of *Pseudomonas* species was reported to be in the 10^8 - 10^9 CFU•mL⁻¹ range, while the KSB and *Rhizobium* species, on the other hand, were in the ranges of 10^4 - 10^5 and 10^6 CFU•mL⁻¹ count, respectively (Table 1). In most cases, there is a significant ($p < 0.0001$) and highest bacterial population observed at 60-days of decomposition, irrespective of the source of animal waste. In addition, our data also confirm that the microbial population of plant beneficial traits exhibits temporal variation in the effects of animal waste source and their decomposition over different days of incubation (Table 1).

The plant beneficial bacteria that appeared were further purified and screened based on their abilities to fix atmospheric N and P-solubilization. We also assume that these PGPB isolates are the most abundant species, given their appearance on their respective culture media after serial dilutions of at least the 10^{-7} range. In this study, 16 different bacteria (e.g., 9 FNFs and 7 PSB) were screened and selected based on their N-fixation or P-solubilization and evaluated further for their performance owing to other plant growth promotion abilities. We observed that 6 FNFs (designated as KP49, KP52N, KP60, KP82A, KP85, and KP113) showed the highest level of N-fixation ($1.05 \pm 0.18 > \text{NH}_4\text{-N} < 2.87 \pm 0.12 \text{ mg}\cdot\text{g}^{-1}$), and the rest 3 bacterial isolates (e.g., KP51, KP109A, and KP109B), however, failed to fix above $1.00 \text{ mg}\cdot\text{NH}_4\text{-N}\cdot\text{g}^{-1}$ of sugar consumed (Figure 1). In the case of the PSB, 4 bacterial isolates, such as KP2, KP52P, KP38, and KP19, show a maximum level of inorganic P-solubilization *in vitro* in both qualitative and Olsen's methods. These bacterial isolates can solubilize inorganic P within the range of $46.39 \pm 6.25 > \text{P}_2\text{O}_5 < 240.69 \pm 18.28 \text{ mg}\cdot\text{mL}^{-1}$ after 14 days of their growth in the culture media (Figure 1). Finally, we report two best-performing PGPB (e.g., KP85 and KP19) among these 16 isolates based on their highest N-fixing and P-solubilization abilities. These data, together, reflect a microbial pool of plant beneficial bacteria and their contribution to the plant nutrient niche of *Kunapajala*.

3.4. *Kunapajala* and derived plant beneficial bacterial isolates are efficient sources of IAA production

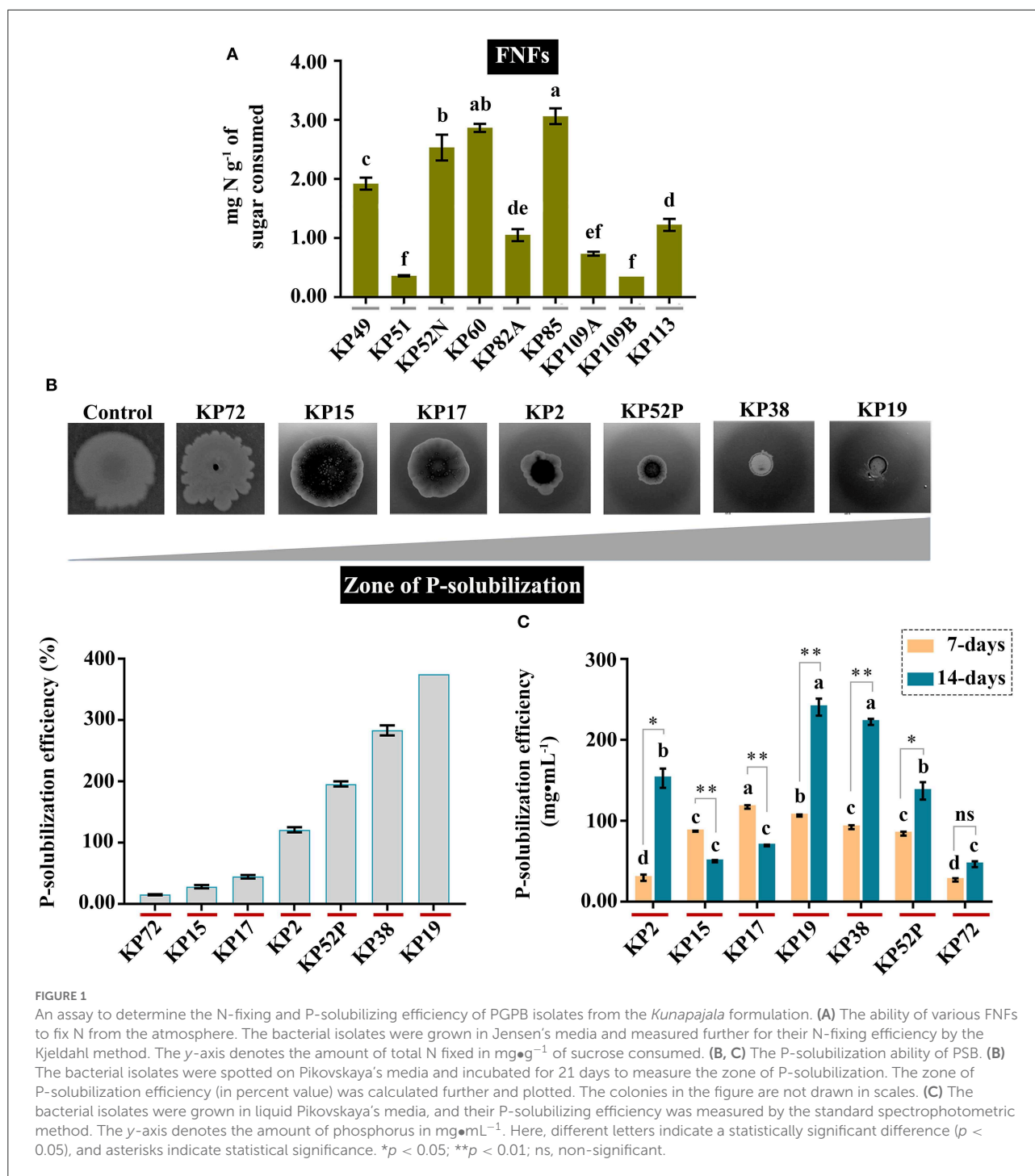
In this study, we report that *Kunapajala* is also a potent source of IAA ($41.00 \pm 1.00 > \text{IAA} < 132.33 \pm 1.53$

$\text{mg}\cdot\text{L}^{-1}$), and the concentration of IAA in the *IKPJ* and *fKPJ* formulations reached the highest peak after 30- and 60-days of incubation, respectively (Figure 2). The dynamic plot revealed that the IAA concentration depletes sharply after 30-days of decomposition in *IKPJ*. In the case of *fKPJ*, however, the concentration of IAA gradually increases up to 60-days of incubation and then declines upon further incubation (Figure 2). Further, we also observed that the IAA concentration and its dynamics are not directly related to the soluble protein content of *Kunapajala* and the population of proteolytic bacteria that decompose proteins into peptides and free amino acids (Supplementary Table A4).

The other source of IAA in the *Kunapajala* formulation could be microbial *in-vivo* IAA synthesis, as bacteria and fungi can also produce IAA besides plants (Spaepen et al., 2007). To elucidate the role of bacteria in IAA production in the *Kunapajala* formulation, we studied the selected FNFs and PSB to evaluate their ability to synthesize IAA. All 16 bacterial isolates (designated KP49, KP51, KP52N, KP60, KP82A, KP85, KP109A, KP109B, and KP113 from FNFs; and KP2, KP15, KP17, KP19, KP38, KP52P, and KP72 from PSB) were confirmed to have indolic compound synthesis ability *in vitro* (Figure 2). However, upon screening 16 different bacterial isolates, there was no bias in the distribution of the magnitude of the IAA-production ability, irrespective of their N-fixation and P-solubilization attributes. These bacterial isolates were studied further for their ability to produce IAA (in $\mu\text{g}\cdot\text{mL}^{-1}$) after 6- and 9-days of growth in LB culture media with $0.10 \text{ g}\cdot\text{L}^{-1}$ of L-tryptophan (see section Materials and methods). It confirms an overall improvement in IAA synthesis (either $p < 0.05$ or $p < 0.01$) after 9-days of growth in most bacterial strains (Figure 2). These results strongly indicate that the bacterial isolates from the class of FNFs and PSB also contribute to the IAA reservoir of the *Kunapajala* formulation.

3.5. Molecular identification of the best FNF and PSB isolated from *Kunapajala*

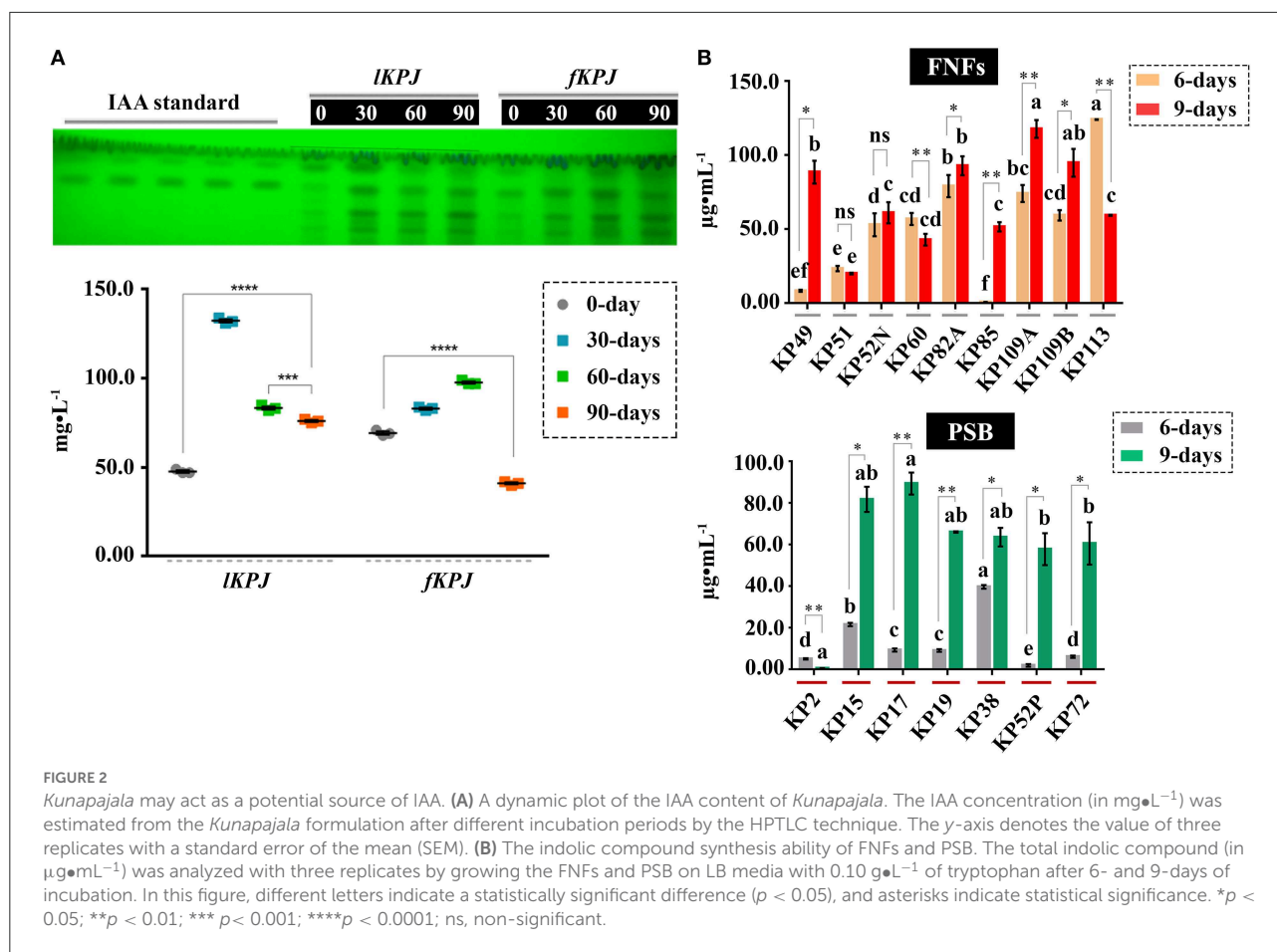
The two best-performing bacterial isolates mentioned earlier were identified further by the 16S rDNA amplicon sequencing technique (Table 2). In this method, the Sanger sequencing reads were obtained for their high-quality sequence coverage by both primers running reversibly. The sequence data thus obtained were joined together to have at least 1.3 kb rDNA sequence coverage by assembling overlapping sequence reads. Finally, the NCBI BLAST program revealed that the best FNF (KP85) and PSB (KP19) show strong sequence homologies with *Pseudomonas chlororaphis* subsp. *aurantiaca* and *Bacillus subtilis* subsp. *subtilis*, respectively (Table 2).



3.6. The biostimulant effects of the *Kunapajala* formulation on rice seedlings: An agronomic and physio-biochemical trait analysis approach

This study discussed the *Kunapajala* technology so far in terms of its roles in waste recycling, contribution to

nutrient cycles, as a source of plant nutrients, plant growth regulators, and plant beneficial bacteria. These observations stimulate immediate follow-up experiments on the biostimulant effects of this technology on the whole-plant and tissue levels, addressing various agronomic and physio-biochemical traits of rice seedlings. Here, we analyzed, in total, 11 agronomic and 3 physio-biochemical traits in rice, including



germination percentage, root-shoot height and weight, yield, total carbohydrates, total proteins, and chlorophyll content. The results revealed a significant difference ($p < 0.0001$) observed among the treatments of the *IKPJ* and *fKPJ* formulations prepared during different decomposition periods (Table 3). We further showed that rice seedlings treated with *Kunapajala* formulations after 60-days of incubation perform better in their crop quality traits, including yield (Table 3). A trend in the dynamics of almost 14 agronomic and physio-biochemical traits in rice, irrespective of animal waste source, reflects a gradient in the plant biostimulant potential of *Kunapajala* formulations at least up to 60-days of incubation. This study, in support, indicates a uniform decomposition pattern and microbial dynamics in both *IKPJ* and *fKPJ*, owing to their combinatorial impacts on plant biostimulant potential. However, the *fKPJ* formulation, in essence, acts better to improve the agronomic traits (Table 3). We next sought to study the plant biostimulant potential of the two best-performing bacterial isolates from the *Kunapajala* formulation. These isolates, alone or in combination, show the best result in crop yield and further quality improvement in rice (Table 3). In conclusion, our study establishes that the *Kunapajala* formulation, in addition to the

derived bacterial strains, could be recommended as a source of plant biostimulants in rice fields.

4. Discussion

4.1. Biodegradable solid waste management and the nutrient potential of *Kunapajala*

The biomass of livestock and fish tissues is in huge global demand as a high-value nutritive source in the human diet. However, the vast amounts of waste generated due to the processing of meat or fish products, either in the fish market or slaughterhouses, respectively, is a growing problem in the urban-based modern world. It recently appeared that 70 and 60% of processed fish and meat products lead to abattoir waste not fit for human consumption (Natalia et al., 2022). The waste management practice generally employs a segregation strategy that converts a substantive portion of this animal waste into the rendering process as poultry or fish feed (Ominski et al., 2021). The remaining waste, in general, is often disposed of in

TABLE 2 Molecular identification of the best performing bacterial isolates reported from the *Kunapajala* formulation.

Bacterial isolate	GenBank accession code	Fragment length (bp)	Closest bacterial strains	Sequence identity (%)
KP85	OM698823	1,384	<i>Pseudomonas chlororaphis</i> subsp. <i>aurantiaca</i>	99.85
KP19	OM698822	1,341	<i>Bacillus subtilis</i> subsp. <i>subtilis</i>	100.00

incineration or landfills, concerning serious hygienic issues and public health. An estimation of over 40 tones of animal waste of daily disposal in India seeks an immediate strategic plan to address the daunting task of solid waste management. In this paper, we have explored the benefits of microbial application in animal waste recycling while revisiting the oldest *Kunapajala* manure technology. Our results have shown that the presence of biomass-degrading bacteria in the *Kunapajala* technology speeds up the animal waste degradation process, causing 10–14% organic C depletion and 25–35% TSP decomposition after 90-days of incubation. To support this further, we also observed an overall consistent enrichment of biomass-degrading bacteria within the 10^7 - 10^8 range (CFU•mL⁻¹) throughout the decomposition period. This bacterial niche is, in fact, likely to release several hydrolytic enzymes (e.g., starch-hydrolyzing, proteolytic, and lipolytic) to favor decomposition (Table 1). In support, *Bacillus subtilis* subsp. *subtilis*, isolated and characterized from *Kunapajala* in this study, has been previously reported to secrete amylases, proteases, and lipases (Latorre et al., 2016; Su et al., 2020). Together, it reflects the microbial degradative capacity in *Kunapajala*, which has turned out to be crucial at the initial stages of the decomposition process. Based on these data, we assume that the *Kunapajala* technology might provide promising applications in animal waste management.

Animal wastes in the *Kunapajala* preparation, as stated in other animal manures (Chen et al., 2019), should include complex biomasses of carbohydrates, proteins, lignin, and fat. Consequently, the hydrolysis of these complex bio-molecular fractions often generates a valuable and concentrated source of water-soluble plant nutrients and biologically stable humic substances, which can be added directly to agricultural fields as soil amendments (Huang et al., 2021). In this study, we report the bio-fertilizer potential of the *Kunapajala* manure in terms of plant-available nutrient conversion recovery and its overall content. In addition, the EC profile in *Kunapajala*, along with neutral pH after 60-days of decomposition, indicates that it may positively stimulate soil processes, biological activity, and overall impact on ecological health when applied to soils (Kumar and Sharma, 2020). This technology, therefore, could provide an eco-friendly pipeline to create a nutrient cycle both *in-situ* and *ex-situ* (Figure 2). The nutrient cycle, in general, is a natural mode of resource management that becomes crucial to balance nutrient recycling and ecosystem health (Harindintwali et al., 2021). Not surprisingly, nutrient

mineralization, mobilization, further uptake by crops, and return to soil organic matter provide a foundation for soil health and sustainable crop cultivation. According to IFOAM-Organics International (https://www.ifoam.bio/sites/default/files/2020-03/poa_english_web.pdf), organic farming practices nurture these naturally occurring cycles and boost their ecological benefits, either through agronomic practices or various improved technologies, to achieve sustainable nutrient management (Lorenz and Lal, 2023). The *Kunapajala* technology as a low-cost valorization approach of animal waste into high-value plant biostimulant may thus appear to act significantly in maintaining soil health and promoting sustainable agriculture.

The safety of the direct application of animal manure to agricultural fields also depends on the toxicity level of heavy metal contamination. Studies predict a high chance of heavy metals cross-transfer to crops, as the concentration above the threshold level could be an alarming issue in animal waste management (da Rosa Couto et al., 2018; Zheng et al., 2022). However, the bio-availability of heavy metals in composts and manures was reportedly lesser in concentration than in anaerobic digestate (Zheng et al., 2022). Therefore, immediate follow-up studies are needed to explore at least two practical aspects of the *Kunapajala* formulation: (i) periodic monitoring of the quality testing parameter of harmful substances, in particular, heavy metal contamination in the *Kunapajala* samples; and (ii) accelerate the decomposition rate of animal waste in combination with physical, chemical, or biological treatments. In continuation, future studies may look at the possibility of the black soldier (*Hermetia illucens*) fly larvae (Lalander et al., 2019; Bortolini et al., 2020) as a biological tool in the *Kunapajala* formulation to speed up the decomposition process.

4.2. The plant biostimulant potential of *Kunapajala* and modes of plant growth promotion

The eco-friendly approach of crop cultivation to improve plant biomass and yield is a top priority worldwide in modern-day agriculture. The use of microbial plant biostimulants, such as the beneficial PGPB, to promote climate-resilient

TABLE 3 The effects of the *Kunapajala* formulation and two bacterial isolates on various agronomic and physio-biochemical traits of rice.

Treatment	Control	<i>IKPJ</i>				<i>fKPJ</i>				FNF and PSB			
		0-day	30-days	60-days	90-days	0-day	30-days	60-days	90-days	KP85	KP19	KP85+ KP19	
GP (%)		64 ± 2.45de	54 ± 2.45e	54 ± 2.45e	74 ± 2.45cd	68 ± 2.00cd	64 ± 2.45de	72 ± 2.00cd	86 ± 2.45ab	70 ± 4.47cd	74 ± 5.10cd	94 ± 2.45a	78 ± 5.83bc
SL (cm)	7 DAS	0.54 ± 0.01g	0.54 ± 0.01g	0.63 ± 0.01f	0.77 ± 0.01d	0.64 ± 0.02f	0.58 ± 0.03g	0.71 ± 0.02e	0.85 ± 0.01c	0.67 ± 0.01ef	0.92 ± 0.02b	0.82 ± 0.03cd	0.99 ± 0.02a
	14 DAS	1.98 ± 0.05h	1.86 ± 0.06h	2.54 ± 0.02ef	2.84 ± 0.04c	2.40 ± 0.08f	2.14 ± 0.07g	2.66 ± 0.04de	3.42 ± 0.07b	2.74 ± 0.04cd	3.46 ± 0.04b	2.70 ± 0.03cd	3.70 ± 0.06a
	21 DAS	2.27 ± 0.01hi	2.25 ± 0.01hi	2.34 ± 0.05gh	2.80 ± 0.03e	2.18 ± 0.06i	2.41 ± 0.05fg	2.52 ± 0.04f	3.94 ± 0.04c	3.05 ± 0.07d	4.21 ± 0.03b	4.13 ± 0.05b	4.50 ± 0.02a
	28 DAS	2.62 ± 0.01h	2.56 ± 0.01h	3.00 ± 0.05f	3.41 ± 0.05d	2.92 ± 0.05fg	2.82 ± 0.05g	2.96 ± 0.02fg	4.15 ± 0.09c	3.18 ± 0.07e	4.44 ± 0.01b	4.42 ± 0.01b	5.01 ± 0.02a
RL (cm)	7 DAS	1.91 ± 0.04f	1.28 ± 0.04h	1.60 ± 0.08g	1.88 ± 0.04f	1.22 ± 0.05h	2.26 ± 0.04e	2.52 ± 0.07d	3.44 ± 0.15a	2.24 ± 0.05e	2.84 ± 0.02c	2.72 ± 0.04c	3.24 ± 0.07b
	14 DAS	1.98 ± 0.05h	1.86 ± 0.06h	2.54 ± 0.02ef	2.84 ± 0.04c	2.40 ± 0.08f	2.14 ± 0.07g	2.66 ± 0.04de	3.42 ± 0.07b	2.74 ± 0.04cd	3.46 ± 0.04b	2.70 ± 0.03cd	3.70 ± 0.06a
	21 DAS	2.99 ± 0.03e	2.25 ± 0.01ij	2.34 ± 0.05hi	2.80 ± 0.03f	2.18 ± 0.06j	2.41 ± 0.05gh	2.52 ± 0.04g	3.94 ± 0.04d	3.05 ± 0.07e	5.14 ± 0.05b	4.72 ± 0.02c	5.52 ± 0.01a
	28 DAS	3.38 ± 0.05h	3.54 ± 0.02gh	3.46 ± 0.04h	4.02 ± 0.10e	3.68 ± 0.02fg	3.50 ± 0.03gh	3.84 ± 0.17f	4.72 ± 0.06d	3.74 ± 0.02f	5.23 ± 0.01b	5.02 ± 0.04c	5.76 ± 0.02a
FW (g)	7 DAS	6.72 ± 0.07f	7.18 ± 0.02e	9.26 ± 0.05c	9.76 ± 0.04a	7.26 ± 0.02e	7.18 ± 0.07e	9.40 ± 0.06bc	9.76 ± 0.07a	7.46 ± 0.07d	9.50 ± 0.04b	9.30 ± 0.06c	9.90 ± 0.03a
	14 DAS	7.50 ± 0.05j	8.44 ± 0.02h	9.62 ± 0.07f	10.48 ± 0.08e	8.64 ± 0.06g	8.58 ± 0.04gh	10.84 ± 0.09d	11.88 ± 0.09a	7.96 ± 0.04i	11.54 ± 0.04b	11.36 ± 0.04c	11.86 ± 0.05a
	21 DAS	8.56 ± 0.07h	9.08 ± 0.09fg	10.86 ± 0.14e	13.20 ± 0.05a	9.12 ± 0.11fg	8.94 ± 0.05g	11.20 ± 0.17d	13.18 ± 0.09a	9.20 ± 0.16f	11.98 ± 0.02c	11.90 ± 0.04c	12.68 ± 0.06b
	28 DAS	9.36 ± 0.07e	9.12 ± 0.06e	11.50 ± 0.19d	13.78 ± 0.20a	9.30 ± 0.15e	9.24 ± 0.13e	11.62 ± 0.15d	13.84 ± 0.22a	9.38 ± 0.15e	12.68 ± 0.04c	12.34 ± 0.12c	13.26 ± 0.08b
DW (g)	7 DAS	1.00 ± 0.03f	1.30 ± 0.08bcde	1.30 ± 0.03bcde	1.30 ± 0.09bcde	1.16 ± 0.11cdef	1.24 ± 0.05bcde	1.38 ± 0.12bc	1.10 ± 0.07ef	1.12 ± 0.04def	1.46 ± 0.02b	1.34 ± 0.06bcd	2.16 ± 0.05a
	14 DAS	1.78 ± 0.05d	1.46 ± 0.02e	1.52 ± 0.05e	1.74 ± 0.14d	1.72 ± 0.05d	1.78 ± 0.05d	1.72 ± 0.05d	1.46 ± 0.09e	1.78 ± 0.05d	2.32 ± 0.04b	2.08 ± 0.05c	2.58 ± 0.04a
	21 DAS	2.34 ± 0.05cde	2.60 ± 0.06bc	2.32 ± 0.10de	2.50 ± 0.16bcd	2.60 ± 0.05bc	2.66 ± 0.05b	2.22 ± 0.07e	2.34 ± 0.15cde	2.66 ± 0.05b	2.54 ± 0.02bcd	2.38 ± 0.04cde	3.12 ± 0.07a
	28 DAS	2.48 ± 0.04f	2.66 ± 0.14ef	2.90 ± 0.08cd	2.92 ± 0.05bcd	2.54 ± 0.05ef	2.72 ± 0.12de	2.90 ± 0.08cd	3.08 ± 0.10bc	2.66 ± 0.07ef	3.12 ± 0.04b	2.94 ± 0.05bc	3.60 ± 0.03a

(Continued)

TABLE 3 (Continued)

Treatment	Control	IKPJ				fKPJ				FNF and PSB			
		0-day	30-days	60-days	90-days	0-day	30-days	60-days	90-days	KP85	KP19	KP85+ KP19	
SL (cm)	45 DAS	13.8 ± 0.97e	16.4 ± 0.51de	18.0 ± 0.63d	26.0 ± 0.63c	19.2 ± 0.58d	23.6 ± 1.96c	23.4 ± 1.03c	37.2 ± 2.27a	31.0 ± 1.10b	37.4 ± 0.75a	37.8 ± 0.37a	38.4 ± 0.60a
	75 DAS	36.6 ± 0.75e	34.2 ± 0.66f	37.0 ± 1.26e	46.6 ± 1.44d	37.4 ± 1.17e	37.8 ± 0.49e	48.8 ± 1.11c	56.4 ± 1.29b	37.8 ± 0.37e	49.4 ± 1.12c	55.8 ± 1.02b	60.0 ± 0.89a
	Harvest	62.6 ± 1.21gh	64.8 ± 1.02g	62.6 ± 0.11gh	83.2 ± 1.39d	62.4 ± 1.08h	74.0 ± 2.10f	76.4 ± 2.09e	85.0 ± 1.41cd	75.6 ± 0.75ef	86.4 ± 1.50c	93.2 ± 1.77b	96.8 ± 1.16a
RL (cm)	45 DAS	7.0 ± 0.55g	10.8 ± 0.49e	11.2 ± 0.37e	13.0 ± 0.63d	10.0 ± 0.32ef	7.8 ± 0.80g	11.6 ± 0.75de	14.8 ± 1.39c	8.6 ± 0.60fg	17.8 ± 0.49b	17.6 ± 0.40b	20.0 ± 0.32a
	75 DAS	14.6 ± 0.40e	14.0 ± 0.32ef	14.2 ± 0.20ef	16.6 ± 0.40d	13.6 ± 0.24ef	11.8 ± 0.58g	15.8 ± 0.37d	18.6 ± 0.40c	13.2 ± 0.20f	20.2 ± 0.20b	20.6 ± 0.24b	22.2 ± 0.37a
	Harvest	18.4 ± 0.40de	18.4 ± 0.40de	18.0 ± 0.32e	20.2 ± 0.20c	20.6 ± 0.24c	16.6 ± 0.24f	19.0 ± 0.32d	22.6 ± 0.40b	20.6 ± 0.24c	24.2 ± 0.20a	24.6 ± 0.24a	24.2 ± 0.20a
NoT	45 DAS	3.4 ± 0.24f	4.0 ± 0.01def	4.4 ± 0.24cde	4.6 ± 0.24cd	3.8 ± 0.20ef	4.2 ± 0.20cde	4.8 ± 0.37c	5.4 ± 0.24b	4.2 ± 0.20cde	5.8 ± 0.20ab	6.0 ± 0.01ab	6.4 ± 0.24a
	75 DAS	8.2 ± 0.20f	8.4 ± 0.24ef	8.6 ± 0.24ef	10.8 ± 0.37c	8.4 ± 0.24ef	9.4 ± 0.24d	10.6 ± 0.24c	11.8 ± 0.20b	9.0 ± 0.32de	12.2 ± 0.37b	13.4 ± 0.24a	14.0 ± 0.01a
	Harvest	11.4 ± 0.40ef	10.8 ± 0.37f	12.4 ± 0.24d	13.8 ± 0.37c	12.2 ± 0.20de	12.4 ± 0.24d	14.0 ± 0.45c	15.6 ± 0.24b	12.0 ± 0.45de	14.0 ± 0.32c	15.4 ± 0.24b	16.8 ± 0.20a
SFW (g)	45 DAS	1.08 ± 0.04g	1.48 ± 0.02e	1.72 ± 0.04d	1.98 ± 0.06c	1.36 ± 0.07f	1.68 ± 0.08d	1.76 ± 0.07d	2.30 ± 0.04b	1.54 ± 0.04e	2.68 ± 0.04a	2.40 ± 0.03b	2.68 ± 0.04a
	75 DAS	4.88 ± 0.06g	5.22 ± 0.04f	5.62 ± 0.05e	6.22 ± 0.04d	5.36 ± 0.02f	5.60 ± 0.04e	6.04 ± 0.06d	6.62 ± 0.07c	5.32 ± 0.04f	7.00 ± 0.05b	7.20 ± 0.19a	7.32 ± 0.06a
	Harvest	14.88 ± 0.06g	15.22 ± 0.04f	15.62 ± 0.05e	16.22 ± 0.04d	15.36 ± 0.02f	15.60 ± 0.04e	16.04 ± 0.06d	16.62 ± 0.07c	15.32 ± 0.04f	17.00 ± 0.05b	17.60 ± 0.06a	17.38 ± 0.23a
SDW (g)	45 DAS	0.37 ± 0.01g	0.45 ± 0.01e	0.42 ± 0.01ef	0.55 ± 0.01b	0.46 ± 0.03de	0.40 ± 0.02fg	0.49 ± 0.01cd	0.61 ± 0.01a	0.52 ± 0.01bc	0.59 ± 0.01a	0.55 ± 0.02b	0.60 ± 0.01a
	75 DAS	0.93 ± 0.02h	1.29 ± 0.01g	1.33 ± 0.01ef	1.41 ± 0.01d	1.28 ± 0.01g	1.34 ± 0.01e	1.46 ± 0.01c	1.54 ± 0.01a	1.31 ± 0.01f	1.49 ± 0.01b	1.45 ± 0.01c	1.55 ± 0.01a
	Harvest	4.85 ± 0.01g	4.77 ± 0.01i	4.81 ± 0.01h	5.20 ± 0.01d	4.74 ± 0.01j	4.86 ± 0.01g	4.96 ± 0.01f	5.10 ± 0.01e	4.78 ± 0.01i	5.30 ± 0.01c	5.33 ± 0.01b	5.40 ± 0.01a
RFW (g)	45 DAS	0.31 ± 0.01f	0.40 ± 0.01ef	0.43 ± 0.01e	0.72 ± 0.01ab	0.47 ± 0.06e	0.51 ± 0.02de	0.58 ± 0.01cd	0.63 ± 0.01bc	0.46 ± 0.01e	0.71 ± 0.05ab	0.65 ± 0.07bc	0.76 ± 0.03a
	75 DAS	2.33 ± 0.02j	2.60 ± 0.01g	2.66 ± 0.01f	2.85 ± 0.01d	2.44 ± 0.01i	2.60 ± 0.01g	2.66 ± 0.01f	2.79 ± 0.01e	2.51 ± 0.01h	2.98 ± 0.01c	3.06 ± 0.05b	3.32 ± 0.01a

(Continued)

TABLE 3 (Continued)

Treatment		Control	IKPJ				fKPJ				FNF and PSB		
			0-day	30-days	60-days	90-days	0-day	30-days	60-days	90-days	KP85	KP19	KP85+ KP19
	Harvest	4.61 ± 0.05h	4.87 ± 0.01fg	4.96 ± 0.01e	5.46 ± 0.01c	4.93 ± 0.01ef	5.10 ± 0.06d	5.17 ± 0.01d	5.39 ± 0.01c	4.84 ± 0.02g	5.77 ± 0.01b	5.85 ± 0.01b	6.18 ± 0.08a
RDW (g)	45 DAS	0.07 ± 0.01e	0.08 ± 0.01de	0.12 ± 0.01cd	0.22 ± 0.01b	0.15 ± 0.02c	0.10 ± 0.01cde	0.13 ± 0.01c	0.23 ± 0.02b	0.11 ± 0.01cde	0.25 ± 0.01ab	0.23 ± 0.03b	0.27 ± 0.01a
	75 DAS	0.80 ± 0.02j	0.90 ± 0.01h	0.96 ± 0.01fg	1.16 ± 0.01d	0.83 ± 0.01i	0.94 ± 0.01g	0.97 ± 0.01f	1.10 ± 0.01e	0.84 ± 0.01i	1.19 ± 0.01c	1.24 ± 0.02b	1.32 ± 0.01a
	Harvest	1.25 ± 0.01gh	1.21 ± 0.01i	1.32 ± 0.01f	1.46 ± 0.01e	1.27 ± 0.01g	1.24 ± 0.01h	1.31 ± 0.01f	1.55 ± 0.01d	1.21 ± 0.01i	1.66 ± 0.01c	1.74 ± 0.01b	1.88 ± 0.01a
TC (mg•g ⁻¹)	45 DAS	86.32 ± 0.94k	108.60 ± 1.23i	124.65 ± 0.63h	181.51 ± 1.16d	147.45 ± 1.02f	101.53 ± 0.60j	110.43 ± 0.15i	174.86 ± 0.66e	135.82 ± 0.22g	196.25 ± 0.91c	211.11 ± 2.05b	222.15 ± 1.30a
	75 DAS	141.97 ± 0.51i	153.74 ± 0.57h	162.10 ± 0.70f	180.83 ± 2.16d	151.75 ± 0.59h	151.65 ± 1.71h	158.71 ± 0.78g	170.17 ± 1.22e	144.39 ± 0.78i	202.51 ± 1.80c	213.51 ± 1.04b	230.49 ± 2.13a
	Harvest	153.43 ± 1.46k	176.79 ± 0.62g	183.50 ± 1.42f	196.41 ± 0.91d	161.18 ± 1.44i	168.19 ± 0.99h	177.95 ± 0.69g	192.05 ± 0.52e	157.87 ± 0.91j	206.29 ± 1.87c	219.87 ± 0.80b	243.86 ± 1.83a
TSP (mg•g ⁻¹)	45 DAS	47.64 ± 0.47h	43.07 ± 1.38i	67.50 ± 1.52e	86.01 ± 0.74c	61.97 ± 1.78f	55.12 ± 0.52g	87.10 ± 0.95c	103.60 ± 1.04a	65.32 ± 0.63ef	77.22 ± 4.18d	84.29 ± 0.63c	97.25 ± 0.50b
	75 DAS	53.84 ± 0.49h	54.37 ± 0.62h	78.40 ± 0.37e	109.59 ± 1.13a	71.86 ± 0.54f	67.60 ± 0.78g	103.41 ± 0.86b	110.19 ± 0.80a	69.43 ± 1.38g	88.30 ± 0.65d	91.46 ± 0.69c	92.97 ± 0.84c
	Harvest	71.52 ± 1.08g	69.76 ± 0.52g	84.24 ± 1.08e	113.81 ± 0.57a	84.75 ± 0.21e	80.31 ± 0.27f	108.66 ± 0.53b	113.81 ± 0.55a	78.92 ± 0.90f	100.90 ± 0.74d	106.90 ± 0.62bc	106.60 ± 0.71c
TChl (mg•g ⁻¹)	45 DAS	10.27 ± 0.02g	7.02 ± 0.14i	6.09 ± 0.24j	13.94 ± 0.18d	10.93 ± 0.19f	9.00 ± 0.09h	11.00 ± 0.05f	17.77 ± 0.53a	12.04 ± 0.11e	15.24 ± 0.29-c	16.99 ± 0.13b	17.06 ± 0.21b
	75 DAS	12.46 ± 0.04f	9.57 ± 0.06g	9.46 ± 0.04g	16.41 ± 0.04cd	13.17 ± 0.22f	15.69 ± 0.50de	17.27 ± 0.54c	20.59 ± 0.47a	14.88 ± 0.27e	17.11 ± 0.18-c	18.12 ± 0.14b	18.93 ± 0.18b
	Harvest	13.26 ± 0.12fg	12.01 ± 1.18gh	10.57 ± 0.14h	16.87 ± 1.36cde	14.73 ± 0.20ef	16.67 ± 0.29de	17.91 ± 0.43bcd	20.82 ± 0.93a	17.34 ± 1.10cd	18.23 ± 0.37bcd	19.11 ± 0.14abc	19.88 ± 0.18ab
NoET		10.4 ± 0.40de	9.6 ± 0.40e	11.4 ± 0.24d	13.2 ± 0.58bc	11.4 ± 0.24d	10.2 ± 0.49de	12.8 ± 0.37c	13.4 ± 0.40bc	11.2 ± 0.49d	13.8 ± 0.37bc	14.4 ± 0.24b	15.6 ± 0.24a
NoG		130.3 ± 0.35j	140.4 ± 0.56i	143.3 ± 0.71h	152.2 ± 0.58e	140.3 ± 0.74i	143.1 ± 0.40h	145.5 ± 0.39g	157.1 ± 0.34d	149.9 ± 0.57f	162.1 ± 0.56c	164.2 ± 0.82b	171.3 ± 0.66a
C:F		0.23 ± 0.01a	0.19 ± 0.01b	0.19 ± 0.01b	0.12 ± 0.01e	0.19 ± 0.01b	0.17 ± 0.01c	0.16 ± 0.01d	0.12 ± 0.01e	0.15 ± 0.01d	0.10 ± 0.01f	0.09 ± 0.01f	0.08 ± 0.01g
TW (g)		13.33 ± 0.12j	14.89 ± 0.16i	16.36 ± 0.27g	19.25 ± 0.09d	15.55 ± 0.03h	17.20 ± 0.21f	18.24 ± 0.09e	19.94 ± 0.16bc	15.07 ± 0.17i	20.30 ± 0.11b	19.59 ± 0.21cd	20.79 ± 0.08a
Y (g)		16.78 ± 0.23h	17.18 ± 0.15h	19.76 ± 0.11f	26.83 ± 0.19d	20.34 ± 0.22ef	18.46 ± 0.21g	20.83 ± 0.41e	26.65 ± 0.43d	19.99 ± 0.10f	29.12 ± 0.25c	30.06 ± 0.18b	30.87 ± 0.17a

Duncan's multiple range test was used to analyze the data, which are represented as the mean ± standard error of samples ($n = 5$), followed by different letters indicating that the difference was statistically significant ($p < 0.05$). The results with the same letters were not significantly different ($p > 0.05$). GP, germination percentage; DAS, date of sowing; SL, shoot length; RL, root length; FW, fresh weight per seedling; DW, dry weight per seedling; NoT, number of tillers per plant; SFW, shoot fresh weight per plant; SDW, shoot dry weight per plant; RFW, root fresh weight per plant; RDW, root dry weight per plant; TC, total carbohydrate; TSP, total soluble protein; TChl, total chlorophyll; NoET, number of effective tillers per plant; NoG, number of grains per panicle; C:F, chaffy: filled grain ratio; TW, test weight; Y, yield per plant.

crop technology has become widely popular in recent years (Du Jardin, 2015; Fadiji et al., 2022). In this study, we showed *Kunapajala* as a potential source of the beneficial PGPB, which includes their roles in N-fixation, P-solubilization, and IAA production, owing to their cumulative beneficial impact on the formulation to crop growth (Figures 1, 2; Table 3). We also report the molecular identification of the best-performing bacterial isolates from the *Kunapajala* source. One of the best-performing isolates, namely *Pseudomonas chlororaphis* subsp. *aurantiaca* is known to inhibit the *in-planta* growth of several important bacterial and fungal phytopathogens (Jain and Pandey, 2016). In addition, these two bacterial species with multiple plant benefits are also non-pathogenic to crop plants and humans (Anderson and Kim, 2018; Su et al., 2020). In this work, we have further established their fitness, alone or in combination, as a biostimulant on rice seedlings. We observed better performance of these bacterial isolates individually or in combination to improve plant performance on yield and other physio-biochemical traits in rice. It seems enigmatic compared to the whole microbial consortium, such as the *Kunapajala* formulation (Table 3). In the microscopic community, the bacterial species often interact with other microbes and form a complex microbial network that adjoins all possible metabolic interactions among microbes, including antagonisms and symbioses (Han and Yoshikuni, 2022). This interactive microbial metabolic network ultimately deciphers various niche-specific ecological functions, including multiple plant-related benefits to crops. The beneficial microbial consortia are known to improve the performance of agricultural crops while maintaining a healthy agroecological niche and the micro-environment. In recent years, the beneficial PGPB have been considered a promising biotechnological tool to improve crop productivity and enhance crop quality traits irrelevant to its nutrient content (Oleńska et al., 2020). Studies also revealed that the active root zone is the site of abundant microbial diversity based on its composition and function (Ray et al., 2020). Therefore, plant-associated microbiome engineering as bio-fertilizers and bio-pesticides has been a sustainable approach, with added benefits in boosting soil biological activity and fertility (Chojnacka et al., 2020; Mitter et al., 2021). These data, together, establish the earlier recommendation of *Kunapajala* as a soil drenching. When applied to soils, microbial communities of plant benefits, in essence, build a mutual relation with niche crops and act cooperatively as a cohort of “healthy-peaceful-societies” to circumvent detrimental fluctuations in crop farming, specifically under challenging conditions.

On the other hand, the microbial consortium often shows a lesser effect, as observed in this study, which may indicate the prevalence of antagonistic interactions over the best-performing single or a few beneficial bacterial isolates. Therefore, the combination of effective strains with better stability of similar genera and additive effects on plant growth promotion often termed synthetic microbial communities (e.g., SynComs),

becomes more robust and is increasingly recognized in modern agriculture (Yin et al., 2022). In this line of evidence, our results strongly encourage the development of an effective bio-fertilizer derived from these two reported bacteria and a few more similarly effective isolates in the near future. Overall, *Kunapajala* technology can integrate plants, microbes, and nutrient relationships to maintain ecological harmony and promote natural resource-based sustainable farming.

4.3. Future perspectives on the scaling-up potential of the *Kunapajala* technology

Kunapajala, as an example, represents an ancient indigenous innovation of India. However, the question remains poised about the practices of such traditional knowledge for adaptation, overall impacts, and vulnerability to the modern economic world. The *Kunapajala* technology provides two main advantages: first, it requires low-cost investment for infrastructural development and encourages the circular bio-economy of agricultural farms; and second, an animal-based agroecosystem may facilitate its sustainable integration to promote inclusive agriculture and rural socio-economic transformation. This study nevertheless also discusses its pivotal role in animal waste recycling. In recent years, the *Swachh Bharat* (Clean India; <https://swachhbharat.mygov.in/>) Mission has been gearing up to generate public awareness and policies about the waste disposal and segregation mechanisms followed by their management into potential applications like energy, fertilizer, animal feed, chemical, and leather industries. The Government of India also launched a flagship initiative, Startup India (<https://www.startupindia.gov.in/>), in order to foster innovative ideas and passion for building up an inclusive ecosystem among entrepreneurs. This industry-academia knowledge exchange platform may aid in planning ideas according to local needs, industrial viability, and national policy plans. The *Kunapajala* technology, in this context, would be more appropriate and socially viable to add up in animal waste management, particularly in the Indian context. In addition, the technological operation of *Kunapajala*, starting with animal waste management to broader applications in agriculture, would also create unique linkages for its strategic implementation, especially in agriculture-based nations.

In India and other parts of the world, organic farming has been growing stupendously as an alternate model of crop cultivation aiming to achieve greater economic viability and environmental sustainability (Meemken and Qaim, 2018; Das et al., 2020). The principle of organic farming practices often emphasizes the safe and efficient recycling of natural resources, including on-farm residues, to counterbalance the antagonistic effects of agrochemicals, maintain ecological harmony, and

conserve biodiversity. In order to substitute the chemical inputs, which are essential resources in conventional agriculture, organic agriculture relies on composts and manures of “true” organic standards and origin. In this study, cow dung and urine, the potential sources of organic matter and microbial consortia, have been collected from the in-house dairy farm, practicing conventional (non-organic) animal farming and therefore fails to qualify as “true” organic manure. Hence, organic animal farming, including poultry and fish, has become an integrated part of this ecological farming system (He, 2020). To monitor the “true” organic standards in complex food chains, the certification process of organic products and farms continues to evolve in the United States and other countries, including India. Therefore, stating terms such as organic manure, organic agriculture, and alike must be carefully considered and discussed. Hence, the *Kunapajala* formulation, often widely coined as organic manure in the past in India, must now qualify for the organic standards and pass through the certification process before being termed organic manure for commercial purposes and scientific agricultural practices.

India is, however, now on the verge of introducing chemical-free natural farming based on on-farm resource recycling and dairy excreta-based microbial formulations (<https://www.niti.gov.in/natural-farming-niti-initiative>). Surely, *Kunapajala* technology would get wider acceptance as a regenerative input under the new Indian agricultural policy. On the other hand, according to the United Nations’ Sustainable Development Goals (<https://sdgs.un.org/goals>), our planet would experience almost 90% of global deforestation primarily due to the expansion of agricultural practices, including crop farming and livestock grazing. It consequently leads to rapid natural resource depletion, agro-pollution, biodiversity loss, unpredictable climate vulnerability, and eventually severe crop loss and yield, having short- and long-term negative impacts on the planet and human health. Therefore, sustainable intensification and ecosystem services in agriculture grab immediate global attention while strengthening the traditional culture and practices to maintain agroecosystem resilience, along with the “inclusive” strategic integration of natural resource utilization and scientific implementation of niche-specific innovations and technologies (Jhariya et al., 2021). For instance, careful animal waste utilization generated from animal farms into recycled animal manure will be far more effective for environment-friendly, sustainable agriculture (He et al., 2020). Therefore, traditional innovations, such as *Kunapajala* technology, could be revisited and assessed to promote sustainable intensification and address ecosystem services across diverse agroecological and socio-economic domains. Hopefully, in the coming years, a more holistic inter-sectoral approach to building a resilience system on the scaling-up potential of *Kunapajala* at par commercial standards would contemplate viable solutions in the socio-economic development encompassing agriculture-environment-public health nexus.

5. Conclusions

In this study, we revisited and assessed the waste recycling potential of *Kunapajala* manures prepared from livestock or fish wastes over different decomposition periods. We have further shown *Kunapajala* to be a dynamic formulation of essential plant primary nutrients and a rich source of PGPB and their metabolic network. This study also elucidates the mechanism of plant growth promotion in *Kunapajala*, including the molecular identification of two plant beneficial bacteria. Based on the 16S ribotyping method, the two best-performing bacterial isolates show strong sequence homology with *Pseudomonas chlororaphis* subsp. *aurantiaca* and *Bacillus subtilis* subsp. *subtilis*. Overall, this work provides the first mechanistic insight into the plant biostimulant potential of *Kunapajala* and its further *in-planta* validation from seed germination to sprouting shoots and roots to grain production in rice.

Data availability statement

The generated nucleotide sequences have been deposited into the NCBI database under the accession 538 numbers: OM698822 (KP19) and OM698823 (KP85).

Author contributions

SM: conceptualization, methodology, validation, investigation, resources, and writing—review and editing. AB and AC: methodology, validation, investigation, resources, and writing—review and editing. RG: methodology, formal analysis, and writing—review and editing. KR, MNA, HB, and JL: formal analysis and writing—review and editing. SS: methodology and resources. AKH and ST: methodology, resources, and writing—review and editing. ASP: writing—review and editing, project administration, and fund acquisition. NR: conceptualization, writing—review and editing, project administration, and fund acquisition. MAA: writing—review and editing and project administration. GC: conceptualization, methodology, formal analysis, investigation, supervision, writing—original draft, project administration, and fund acquisition. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fsufs.2022.1073010/full#supplementary-material>

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