MEMORANDUM OF UNDERSTANDING
BETWEEN

SCHOOL OF BIOTECHNOLOGY AND BIOINFORMATICS, D.Y.PATIL DEEMED TO BE UNIVERSITY, NAVI MUMBAI, MAHARASHTRA

AND

HINDI VIDYA PRACHAR SAMITI'S RAMNIRANJAN JHUNJHUNWALA COLLEGE (AUTONOMOUS), MUMBAI, MAHARASHTRA

In compliance with the prevailing rules and regulations, a research & development collaboration between **Hindi Vidya Prachar Samiti's Ramniranjan Jhunjhunwala College of Arts, Science and Commerce**, Mumbai, Maharashtra and **School of Biotechnology and Bioinformatics**, D.Y. Patil Deemed to be University, Navi Mumbai, Maharashtra, India.

Both the signatories to the agreement are adequately authorized statutorily to executive this agreement and bind the respective organizations that aim to co-operate in research and development.

Whereby, it is now agreed between **Hindi Vidya Prachar Samiti's Ramniranjan Jhunjhunwala College of Arts, Science and Commerce**, Mumbai, and **School of Biotechnology and Bioinformatics**, D.Y.Patil Deemed to be University, Navi Mumbai as under:

a) The **School of Biotechnology and Bioinformatics**, D.Y.Patil Deemed to be University, Navi Mumbai will acknowledge R J College in research articles published related to work conducted in the laboratories of Department of Biotechnology, R J College, Mumbai.

b) The **Ramniranjan Jhunjhunwala College of Arts, Science and Commerce**, Mumbai will provide infrastructure facilities for research work.

c) This MOU will be valid from a period of June 2018 to May 2022.

d) Parties are at liberty to revoke/ cancel this understanding by giving 30 days notice.

e) Revision of this mutual agreement shall be applicable at any time through the official request of either of the two parties and with a due consideration of mutual terms and conditions.

IN WITNESS WHEREOF, The parties hereto have offered their signatures:

Dr Usha Mukundan
Principal
Hindi Vidya Prachar Samiti's
Ramniranjan Jhunjhunwala College
of Arts, Science and Commerce

R. J. College of Arts, Science & Commerce, Est. 1963, AUTONOMOUS, NAAC Re-accredited 'A' Grade, Ghatkopar (W), Mumbai-400 086, INDIA

Place: Mumbai
Date :

Dr Debjani Dasgupta
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Isolation and screening of microorganisms from traditional and modified agro-organic formulations

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Abstract

Agriculture is an important sector contributing to the Indian economy. Adopting eco-friendly approaches should be preferred over chemical fertilizers due to their cost-effectiveness, better crop productivity, and sustainability. The use of native organic preparations like Panchagavya and Jeevamrit which are rich in macronutrients, micronutrients, plant growth-promoting substances, and beneficial microorganisms are proved to be promising in achieving sustainable agriculture. The objective of this study is to isolate and characterize the plant growth-promoting abilities of the microorganisms from the traditional and modified formulations prepared using agro-organic waste. Four different formulations viz., traditional and modified Panchagavya and Jeevamrit were respectively prepared. The microbial population present in these formulations was evaluated for microbial load by enumerating the number of bacteria, fungi, and actinomycetes at different time intervals of preparation. Among the traditional formulations, the highest microbial population was observed in Jeevamrit (19.1×10^8 CFU/ml) followed by Panchagavya (3.04×10^8 CFU/ml). While for modified formulations, the highest microbial population was observed in modified Jeevamrit (11.41×10^8 CFU/ml) followed by modified Panchagavya (1.72×10^8 CFU/ml). The study also attempted to estimate, the percentage of nitrogen fixers and phosphate solubilizers in both traditional and modified formulations with respect to time. A common trend of increasing percentage of nitrogen fixers and phosphate solubilizers was observed in all the prepared formulations. A total of 105 different microbial isolates were isolated. For isolating bacteria from this agro-organic preparation, nystatin (50 mg/l) was used to inhibit the growth of fungus. Among the 105 isolates, the Gram character of the 68 bacterial isolates was confirmed by staining and non-staining (KOH) methods. The isolates were further screened qualitatively for different metabolic characteristics like nitrogen fixation, phosphate solubilization, starch hydrolysis, cellulose, and pectin degradation. Of these, 11 bacterial isolates showing plant growth-promoting activities like atmospheric nitrogen fixation and phosphate solubilization were selected for further studies.

Key words: Organic formulations, Panchagavya, Jeevamrit, nitrogen fixers, phosphate solubilizers, plant growth promotion.

Introduction

The green revolution fulfilled the demand for increased yield for the growing population, but it also increased the use of chemical fertilizers in agriculture. Improved agricultural productions lead to a decrease in soil fertility which is one of the most important constraints faced by the agricultural sector [1]. Globally, the adoption of eco-friendly agriculture practices for sustainable agriculture is required. Organic farming is an ecologically comprehensive system growing worldwide for increasing sustainability as it is an alternative for conventional agriculture [2]. It has been practiced in developing countries like India since ancient times and also supports the crop yields and quality of production by increasing the microbial diversity of the soil [3,4]. Organic farming is mostly dependent on the natural microflora of soil and ensures safe food for human consumption [5]. Liquid organic manures are becoming popular and are an abundant source of nutrients, growth stimulants and bio-control agents. They can be prepared using various materials available locally, farm inputs and daily household materials. Thus, the cost of preparation decreases in comparison to conventional fertilizers [6]. Panchagavya and Jeevamrit are cow dung-based liquid manures containing an enormous amount of beneficial microbial load. The use of these fermented products is gaining attention among farmers and research communities aiming to improve soil sustainability and reduce the cost of cultivation. They are said to be potent sources of macro and micronutrients, plant growth-promoting factors, and also immunity enhancers [7,8]. It has been reported that the combined application of Panchgavya and Jeevamrit with other organic manures has increased dry fruit, chili, and rice yields [9,10]. The plant growth-promoting bacteria present in these naturally fermented products act as biocontrol agents [11]. To familiarize the use of Panchagavya and Jeevamrit it is necessary to scientifically validate the physical, chemical, and biological parameters. With this stimulus, this study focuses on isolating potent plant growth-promoting bacteria specifically able to fix atmospheric nitrogen and phosphate-solubilizers which would be further used for preparing bacterial consortium.

Materials and Methods

Preparation of organic formulations: Both Panchagavya (PVG) and Jeevamrit (JVM) were prepared as per the method by Kumar *et al.* [12]. Modified organic formulations (MPVG - Modified Panchagavya, MJVM - Modified Jeevamrit) were prepared by adding agro-organic waste and fermented for respective time duration.

Enumeration of microbial population in the formulations: The fermented samples from prepared formulations were withdrawn at different time intervals. Enumeration of microbial population in traditional and modified samples was analyzed (Table 1).

Table 1. Enumeration of microbial population in traditional and modified samples.

Time (T)	Days
Traditional	(PVG & MPVG) samples
T1	12
T2	19
T3	26
Modified	(JVM & MJVM) samples
T4	7
T5	14
T6	21

Standard plate count (SPC) of all the six samples was performed to compare the conventional and modified organic formulations on the total microbial population viz. bacteria, fungi, and actinomycetes. The samples were suitably diluted and plated on nutrient agar, Sabraoud's agar, Kuster's medium and incubated at 28-30°C for 24-48 h.

***Isolation of bacteria from the formulation mixture*:** Different bacterial strains were isolated from the microbial mixture using nystatin (50 mg/l) as an antifungal agent [13]. The isolated strains were further characterized based on their biochemical characteristics and Gram character by the staining and non-staining (KOH) method [14]. All the bacterial isolates were maintained on nutrient agar slants at 4°C and in 50% glycerol stock at -80 °C for future use.

Screening of isolates for different metabolic characteristics: The bacterial isolates were screened for nitrogen fixation on Waksmann No 77 medium [15], phosphate solubilization on Pikovskaya's medium [16], starch hydrolysis on starch agar (2%) [17], cellulose degradation on cellulose minimal agar [18], and pectin degradation on Vincent's agar (pH 7.0) [19]. Following incubation of plates at 30°C the microbial counts were taken after 24 h and expressed as CFU/ml.

Results and Discussion

Effect of fermentation time on microbial population in traditional and modified formulations: Figs 1 and 2 show the highest microbial population observed in T1 (i.e. after 12 days of fermentation) for PVG and MPVG. The highest value for microbial population noticed in PVG is bacteria (17.3×10^9 CFU/ml), fungi (12.8×10^9 CFU/ml) and actinomycetes (3×10^{10} CFU/ml); while for MPVG is bacteria (15.7×10^9 CFU/ml), fungi (1×10^9 CFU/ml) and actinomycetes (5×10^{10} CFU/ml).

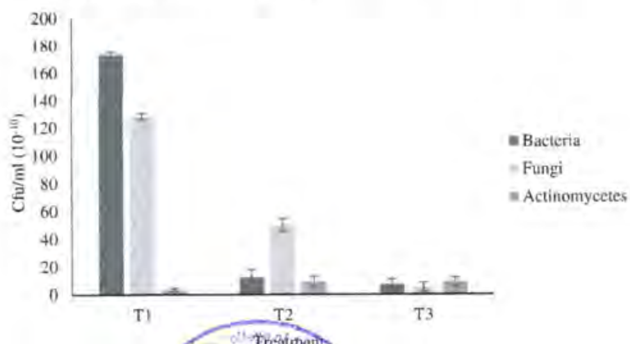

Figure 1. Analysis of the microbial population in PVG.

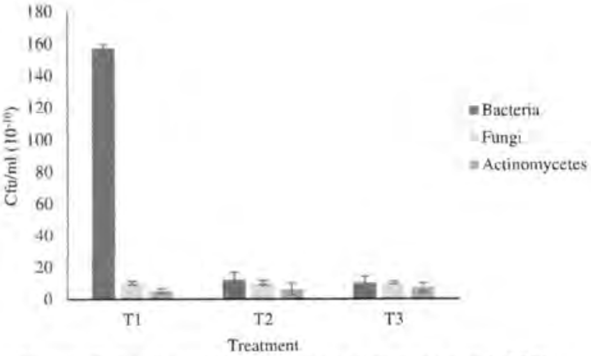

Figure 2. Analysis of the microbial population in MPVG.

In PVG, the bacterial and fungal population is observed to be decreasing while the actinomycetes population is increasing with time. In MPVG, the bacterial population is decreasing while the fungal and actinomycetes population is increasing at a slower pace with time. The ingredients used for making PVG and MPVG may have an abundance of bacterial flora inherently or either be rich in nutrient source causing the increase in bacterial population on the 12th day of fermentation. Cow dung-based biodynamic preparations have an abundance of the bacterial population[20]. The content was stirred twice a day to maintain the aerated conditions, which might lead to a shift in the bacterial population and supporting the growth of the fungal and actinomycetes population with increasing days of fermentation.

Figs 3 and 4 show the highest microbial population in T4 (i.e. after 7 days of fermentation) for JVM and MJVM, respectively. The highest value for microbial population noticed in JVM is bacteria (8.9×10^8 CFU/ml), fungi (5×10^8 CFU/ml) and actinomycetes (4×10^9 CFU/ml); while for MJVM is bacteria (2.9×10^8 CFU/ml), fungi 4.81×10^8 CFU/ml) and actinomycetes (3.7×10^9 CFU/ml). The abundance of microbial population is a result of active soil being used during preparation serving as an inoculant for the survival of beneficial microorganisms [22].

In JVM, the bacterial population is observed to be decreasing with time. whereas fungal population showed a decrease on the 14th day of fermentation but an increase was observed with increasing time possibly due to pH change. The actinomycetes population showed an increase on the 14th day of fermentation, whereas the decrease was observed over time. In MJVM, the bacterial and fungal population was observed to be decreasing with time, whereas actinomycetes population showed a similar trend as in JVM. Devakumar *et al.* [23] noticed a similar trend of decrease in bacteria and fungi load in Jeevamrit.

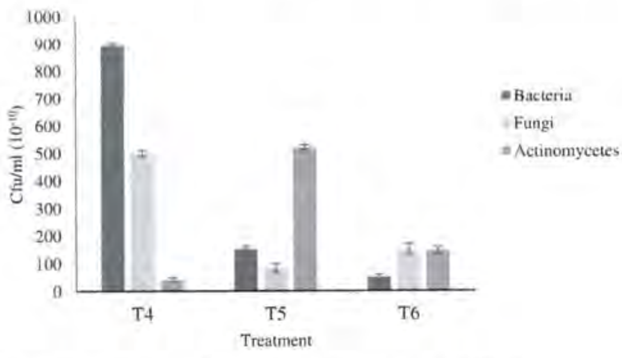

Figure 3. Analysis of the microbial population in JVM.

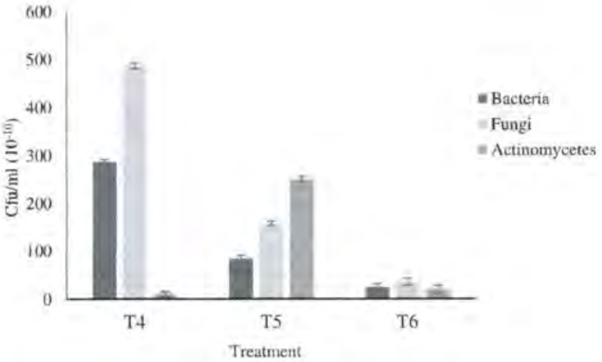

Figure 4. Analysis of the microbial population in MJVM.

Traditional formulations were found to have a higher microbial population as compared to modified formulations. Among the traditional formulations, the highest microbial population was observed in JVM (19.1×10^{8} CFU/ml) followed by PVG (3.04×10^{8} CFU/ml). Chakraborty and Sarkar [6] also noticed that Jeevamrit has a higher microbial load followed by Panchagavya [21]. Plant growth-promoting traits like phosphate solubilization and nitrogen fixation are reported to play an important role in growth promotional effect[24]. Thus, the percentage of nitrogen fixers and phosphate solubilizers was estimated in both traditional (PVG & JVM) and modified (MPVG & MJVM) formulations with respect to time.

Fig.5 represents the highest percentage of nitrogen fixers at T3(53%) and T4(92%) for traditional formulations; while T3(73%) and T4(80%) for modified formulations. Fig. 6 represents the highest percentage of phosphate solubilizers at T3(47%) and T5(87%) for traditional formulations; while T5(90%) for modified formulation (MJVM). No phosphate solubilizers were observed in MPVG for the study period. The presence of cow urine as a preparation ingredient is possibly stimulating the growth of nitrogen fixers and phosphate solubilizers in the fermented product[25]. The population of nitrogen-fixing and phosphate solubilizing bacteria is affected by the breakdown of organic waste during fermentation [26].

In this study, a rise in nitrogen-fixing and phosphate solubilizing organisms was observed with time. The higher microbial load found in the prepared organic formulations is in conformity with other authors [25, 27, 28] reporting the presence of beneficial microorganisms in liquid manures. The organic formulations contain special

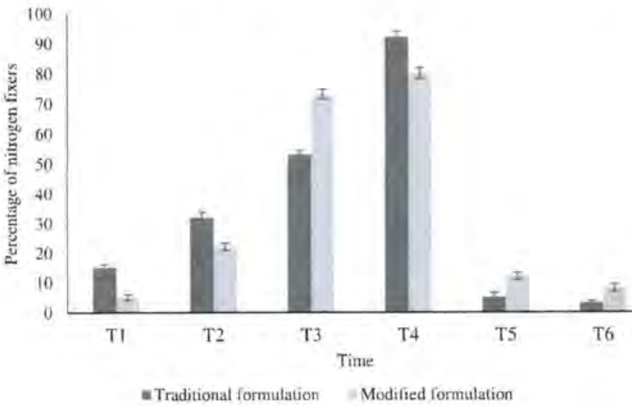

Figure 5. Atmospheric nitrogen fixers in traditional and modified formulations.

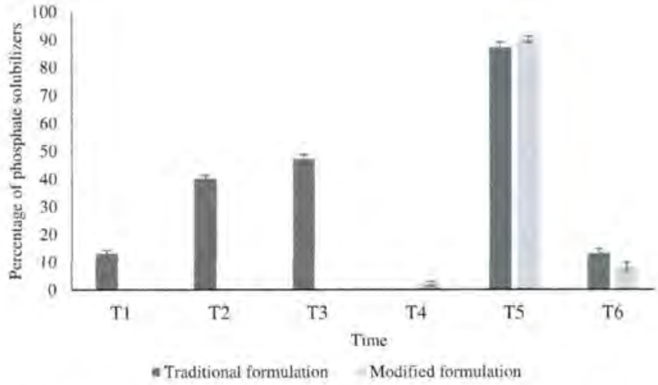

Figure 6. Phosphate solubilizers in traditional and modified formulations.

biochemical groups of microorganisms like nitrogen fixers, phosphate solubilizers in addition to general microflora. Hence, these organic formulations could be used in supplementing the quality of crop production.

Isolation of bacteria from prepared organic formulations: The organic formulations showed an abundance of microbial communities, and about 105 different microbial cultures were isolated. Among these, 68 bacterial cultures were differentiated from the total microbial isolates using nystatin as an antifungal agent. Pure cultures were obtained by using streak plate method[29], these isolates were also checked for different functional diversities like starch hydrolysis, nitrogen fixation, phosphate solubilization, cellulose, and pectin degradation.

In this study, 11 different isolates from the pool of 68 bacterial cultures were further checked for their microscopic and metabolic characteristics. Table 2 summarizes the results for metabolic characteristics of selected isolates. The diazotrophic character of the isolates was evaluated by checking the capability to grow in a nitrogen-free selective medium. All the isolates could fix nitrogen; while isolates 2, 4, 5, 6, and 11 could solubilize phosphorus from

Table 2. Metabolic characteristics of the isolates.

Isolate	Nitrogen Fixation (a)	Phosphate Solubilization (b)	Starch Hydrolysis (c)	Pectin degradation (d)	Cellulose degradation (e)
1	+	-	-	-	-
2	+	+	-	-	-
3	+	-	+	-	-
4	+	+	+	-	-
5	+	+	-	+	-
6	+	+	+	-	+
7	+	-	-	-	+
8	+	-	+	-	-
9	+	-	-	-	+
10	+	-	+	-	-
11	+	+	-	-	+

Key: (a) Nitrogen fixation: (+) growth observed. (b) Phosphate solubilization: (+) halo zone produced after phosphate solubilization (c) Starch hydrolysis: (+) zone of clearance after starch hydrolysis (d) Pectin degradation: (+) zone of clearance (e) Cellulose degradation: (+) zone of clearance insoluble tricalcium phosphate. The starch hydrolysis and pectin degradation ability were exhibited by isolates 3, 4, 6, 8, 10, and 5 respectively; while isolates 6, 7, 9 and 11, could degrade cellulose.

Table 3 summarizes the results for microscopic characteristics of selected plant growth-promoting bacteria by performing Gram's staining and the results were confirmed by performing non-staining methods using 3% KOH solution.Based on microscopic examination using Gram's staining and rapid test (KOH) for verifying the Gram's staining performed [30], the 11 selected isolates are confirmed as bacteria in morphology.

Table 4. Microscopic characteristics and non-staining (KOH) tests for the isolated strains.

Strain	Microscopic morphology	KOH test
1	Gram-positive rods	-
2	Gram-positive rods	-
3	Gram-negative rods	+
4	Gram-positive cocci	-
5	Gram-negative coccobacillus	+
6	Gram-positive coccobacillus	-
7	Gram-negative rods	+
8	Gram-positive cocci	-
9	Gram-negative rods	+
10	Gram-negative rods	+
11	Gram-negative rods	+

Key: KOH solubility test: (-) Gram-positive organism; (+) Gram-negative organism

Conclusions

The microbial population present in the prepared organic formulations was evaluated with respect to time for a month and a common trend of increasing percentage of nitrogen fixers and phosphate solubilizers was observed. A total of 105 microbial cultures were isolated, among these 68 bacterial cultures were differentiated based on their functional diversities. For further studies, 11 bacterial isolates were found to be exhibiting specific plant growth traits like nitrogen fixation and phosphate solubilization and thus, would be used for making consortium and checking its effect on plant growth.

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Author's Contributions and No Conflict of Interest

All three authors have designed the experiment, wrote the main manuscript text along with analysis of data. All the authors do not have any conflict of interest for this research data.

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