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MICROBIOLOGICAL INTERVENTIONS FOR IMPROVEMENT OF TASAR HOST PLANTATION ECOSYSTEMS

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ABSTRACT

Tasar host plants are trees with deciduous leaf fall patterns showing heavy leaf fall during autumn and consistent leaf fall at a lower rate during other seasons of the year. The leaf biomass is regenerated during spring which is subsequently fed to the silkworms. The trees require additional nutrient uptake to regenerate the lost biomass. The current research focuses on increasing the nutrient availability to the host plants through microbiological interventions in the form of microbial consortia for degrading and mineralizing the tree litter and symbiotic bioinoculants to improve nutrient use efficiency. The shred leaf biomass of the tasar host plants had an average nitrogen content of 1%. The leaf litter was allowed to degrade under different moisture levels and the microbial population and diversity were studied. A total of 29 bacteria and 10 fungi have been isolated from decaying leaf litter of arjun, asan and sal which will further be evaluated for degradation efficiency and will be utilized in the form of consortia to accelerate in situ degradation and composting/ vermicomposting processes. Ectomycorrhiza are a type of mycorrhiza which form symbiotic associations exclusively with roots of trees and provide them with N, P and water in exchange to the sugars required by the fungi for their growth. Tasar growing areas are very congenial for ectomycorrhizal associations and the natural sal forests have a wealth of such associations. A total of 110 types of fungal fruiting bodies have been collected from the forests of Chaibasa, Chakradharpur, Kharsawan and tasar host plantations as well as sal forest of CTR&TI, Ranchi. The microscopic observations revealed the wide variation in hyphal structure, sporulation patterns and morphology of spores. These will be further screened for their symbiotic and bioinoculant potential. These microbiological interventions enable to improve the productivity of the tasar host plantation ecosystems in a sustainable manner.
Keywords: *Terminalia arjuna*, *Terminalia tomentosa*, *Shorea robusta*, Ectomycorrhizal fungi, Litter degradation.

Introduction

A plant growing in a natural environmentis in itself a composite community (Lundberg *et al.*, 2012). A well-structured and regulated community of microorganisms is always associated with the plant-the plant microbiome (Chaparro *et al.*, 2014; Lebeis, 2014; Bulgarelli *et al.*, 2015). Although the plant is a single organism, the soil-plant system comprises of different microhabitats like phyllosphere, phylloplane, carpoplane, rhizoplane, rhizosphere, spermosphere, etc., which vary widely with respect to abiotic and biotic characteristics (Sivakumar *et al.*, 2020). The structure of the microbial community in such microhabitats is affected by the physiological performance of the plant and vice versa. The plant microbiome can be visualized as an important part of plant body regulating the physiology and thereby productivity of plants. Engineering the soil-plant microbiome is an important tool in modifying the physiology of the plants in order to improve the plant productivity in a sustainable manner. Microbial interventions can be categorized into direct or indirect methods of altering the microbial community structure and function of the soil as well as plant. The direct method involvesthe use of microbial bioinoculantswhereas the indirect method involves enriching the autochthonous population through fortification and augmentation (Dhaliwal *et al.*, 2022).

Tasar sericulture is a system of rearing the silkworms in the wild i.e., on the host plants directly. Tasar host plants are large trees and raised in plantations to carryout sericulture. They are characterized by high nutrient requirements driven by the necessity to regenerate the biomass corresponding to their leaf loss due to feeding by the silkworms. In view of developing sustainable crop production practices, the productivity of the tasar host plants can be improved through efficient nutrient recycling in the soil and by increasing the nutrient use efficiency of the plants using microbiological means. The tasar host plants exhibit deciduous leaf fall pattern and thus a huge quantity of leaf litter is generated. Under natural conditions, the litter degradation processes are slow and the rate of nutrient loss through volatilization and leaching are higher than the rates of mineralization and immobilization. Since the litter remains on the surface for longer periods it is prone to displacement through natural as well as anthropogenic activities. Specially designed microbial consortia can be employed to enhance the rate of litter degradation and streamline the nutrient recycling processes in the tasar ecosystems.

The tasar sericulture areas majorly comprise of acidic and low structured soils reducing the nutrient use efficiencies of the host plants. The conventional microbial technologies like PGPR based biofertilizers used for crop plants are not sufficient to enhance the nutrient use efficiencies of the tree plantations. Robust symbiotic partners are required to bring significant upgradation in the nutrient uptake processes of the trees. Ectomycorrhiza are fungal symbionts which form mycorrhiza exclusively with tree species. They exhibit P uptake efficiency similar to the VAM and also benefit the plants with improved N and water uptake. Moreover, the soil conditions of the tasar host plants are more favourable for the formation and maintenance of ectomycorrhizal symbioses (Huey *et al*., 2020).

The microbial interventions initiated for the improvement of tasar host plant productivity including studies on litter degradation with an attempt to design a microbial consortium for accelerating in situ degradation of the leaf litter under low moisture conditions and the diversity of fungal communities,suspected with ectomycorrhizal potential, of forests harbouring the tasar host plants *Shorea robusta*, *Terminalia arjuna* and *T. tomentosa* in order to subsequently develop ectomycorrhizal bioinoculants for tasar host plant improvement.

Materials and Methods

Studies pertaining to leaf litter degradation

Sample collection:

Leaf litter was collected from three different tasar host plants - Arjun (*Terminalia arjuna*) and Asan (*Terminalia tomentosa*) of CTR&TI farm and Sal (*Shorea robusta*) of the forest patch adjacent to the campus of CTR & TI.

Texture observation:

The strength and texture of the dried leaf were assessed by physical observation.

Experimental set up for litter degradation study:

An experiment was set up to study the microbiology of the litter degradation under low moisture conditions. Leaf litter of Arjun, Asan, Sal and a mixture of equal proportion of all three, weighed to 6g were taken in the 250 ml conical flask. Sterile water was added to the litter suitably to maintain the moisture at 10% and 30%. The weight of the flasks was recorded and incubated for two weeks. The moisture levels were maintained by checking the loss of weight of the flasks regularly.

Enumeration and Diversity of microbial population:

Bacterial and fungal population was estimated through culture-based method- total plate counts, on tryptone soy agar and Sabouraud's dextrose agar respectively (Atlas, 1995). The total number of morphotypes of bacteria and fungi in each sample are represented as species richness.

Nitrogen estimation: The total nitrogen content of the leaf litter of the three host plants was estimated through digestion and Kjeldahl distillation method (Krik, 1950).

Statistical analysis: The population studies are done in triplicate and results are expressed as mean ± Standard error of mean.

Studies on diversity of fungal fruiting bodies of tasar growing areas

Collection of fungal fruiting bodies:

Fungal fruiting bodies have been collected from the forests of Chaibasa, Chakradharpur, Kharsawan and tasar host plantations as well as sal forest of CTR & TI, Ranchi. The collected fungal fruiting bodies were washed, surface sterilized and inoculated on Sabouraud Dextrose Agar (SDA) with ampicillin. The fungi were purified and maintained on SDA.

Study of Fungal Morphology:

The fungi were grown through slide culture method and stained with lactophenol cotton blue. The morphological characters were recorded through light microscopy.

Results and Discussion

Litter Properties

The data indicates that sal has coarse leaf litter characters which delay the decomposition processes but has a higher nitrogen content compared to arjun and asan. The results indicate that the overall nitrogen content of litter is lower and nitrogen addition may increase the rate of litter degradation. Martínez-García *et al*. 2021 showed that Nitrogen content of litter is directly correlated with degradation rate.The physical and chemical properties of the leaf litter of different plants under study are presented in Table 1.

Table 1: Properties of leaf litter of the primary host plants of Tasar silkworm.

Property	*Shorea robusta*	*Terminalia arjuna*	*Terminalia tomentosa*
Thickness	High	Low	Low
Fragility	Low	Moderate	Moderate
Total Nitrogen (%)	1.34	1.00	0.75

Microbial Population and Diversity

The population of bacteria and fungi as well as diversity in terms of species richness during degradation were recorded in dried sal, arjun, asan and a mixture of these leaves at different moisture levels. The results specify that the population of bacteria of dried leaves of sal is generally lower than that of arjun or asan. Yakimovich *et al*. 2018 reported a dominance of fungal population over bacterial population in litter having higher polyphenol content. Sal leaves are reported to have high polyphenol content and thus the composition of microflora of sal litter is in accordance with the previous reports.

In general increase in moisture level increased the population and species richness of bacteria as well as fungi, whereas arjun is an exception with no considerable difference in the microbial population and diversity at the two moisture levels studied (Table 2). Bacteria grow profusely at inherent moisture levels of 90% and above whereas fungi are comparatively more xero tolerant and can grow at inherent moisture contents of 70% and above. Thus, at low moisture levels, the population densities of the litter are low and tend to increase with increasing moisture content. An increase in litter decomposition rate from 5 to 34 % was reported due to wetting by Cisneros‑Dozal *et al*., 2007.

Table 2: Population and species richness of bacteria and fungi in degrading leaf litter at different moisture levels.

Sl. No.	Sample	Moisture Level (%)	Bacteria Population CFU/g leaf± SEM	Bacteria Species richness	Fungi Population CFU/g leaf± SEM	Fungi Species richness
1	Sal	10	50± 2.6 X 10^3	1	12 ± 1.3 X 10^4	4
2	Sal	30	23± 1.2 X 10^4	7	70± 2.6 X 10^4	10
3	Arjun	10	21± 0.7 X 10^5	7	20± 1.2 X 10^3	4
4	Arjun	30	18±0.9X 10^5	9	15± 0.9 X 10^3	4
5	Asan	10	35± 1.0 X 10^4	6	10± 1.0 X 10^3	3
6	Asan	30	65±1.2 X 10^4	10	20 ± 0.7 x 10^3	5
7	Mixed	10	60±0.8 X 10^4	7	20 ± 0.8 x 10^3	5
8	Mixed	30	25 ± 1.2 X 10^5	10	40± 1.3 x 10^4	7

In sal litter, the fungal population was higher than the bacterial population at both the moisture levels. Highest fungal diversity was observed in sal litter whereas highest bacterial diversity was observed in asan. This again can be attributed to the polyphenol content. Shifts in microbial communities to fungal dominance with respect to increasing polyphenol content has been reported (Yakimovich *et al.*, 2018). The mixture of all the leaves showed population as well as density in similar ranges as the parent material due to contribution from various litter. A total of 29 bacterial isolates (figure 1) and 10 fungal isolates involved in litter degradation were isolated, purified and maintained.

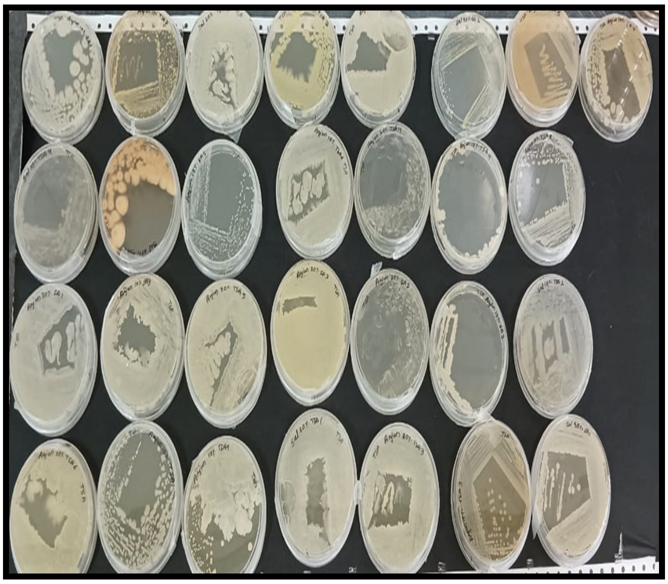

Fig. 1: Bacterial isolates with litter degradation potential

Diversity of fungal fruiting bodies:

A total of 110 fungal fruiting bodies have been collected from the study areas which exhibits the great abundance of these fungi in the forests and tree plantation ecosystems of these areas. Examination of the characters of fungal fruiting bodies and the morphology of fungal cultures show that the tasar host plant ecosystems harbor a wide variety of fungi. Although many fungi are capable of growing on decaying matter and ant or termite mounds producing fruiting bodies, in the current study the fruiting bodies growing in soil in the vicinity of trees were collected and have a very high probability of having ectomycorrhizal potential. The abundance and species richness of fungal fruiting bodies varied widely from place to place as illustrated in figure 2. The microscopic observation revealed the hyphal morphology which varied morphologies like segmented, continuous, highly granular protoplasm, etc. Some fungi exhibited existence of more than one type of hyphae indicating the possibility of fungal gametophore formation. Characteristic asexual as well as sexual spores have been observed (figure 3). Most of the fungi forming fruiting bodies belong to ascomycetes and basidiomycetes and possess unique spore characteristics (Hawksworth, 2007). Ectomycorrhiza belonging to different species of *Russula*, *Lactairius* and *Amanita* have been previously reported from the Sal forests of Madhya Pradesh and Chhattisgarh, India (Sharma *et al.*, 2008; 2009a; 2009b)

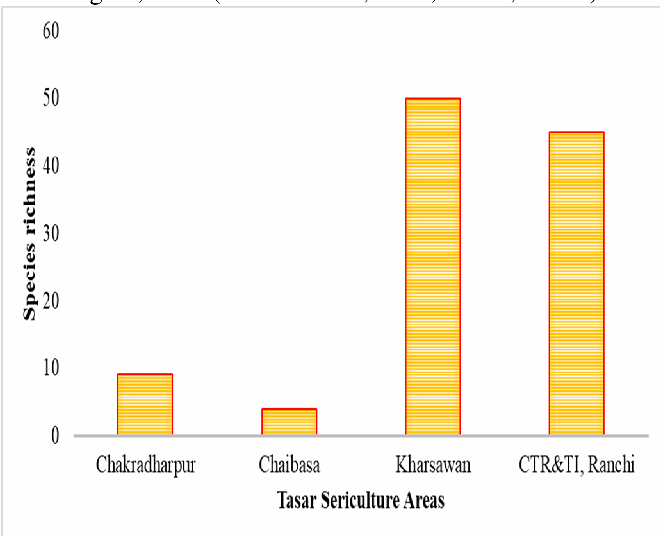

Fig. 2 : Species richness of fungal fruiting bodies in different tasar sericulture areas

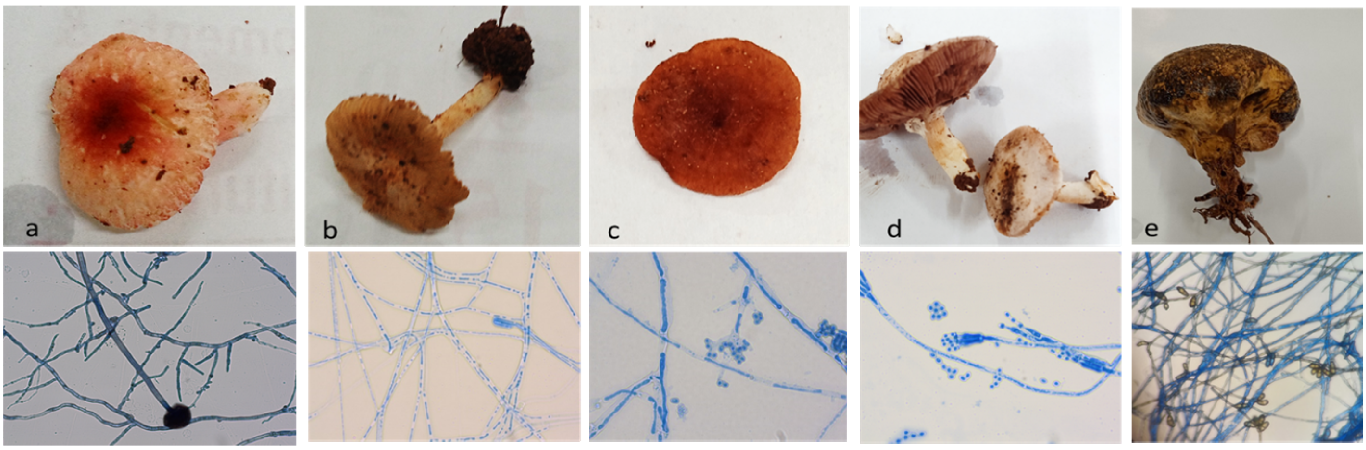

Fig. 3 : Morphological features of different fungi isolated from tasar sericulture areas

Conclusion

The different morphotypes of bacteria and fungi from different samples were purified and maintained as axenic cultures. A total of 29 bacterial isolates and 10 fungal isolates involved in litter degradation were preserved and maintained. The population densities of microbial community in degrading litter are positively influenced by moisture content and negatively influenced by the polyphenol content of the litter. The bacteria and fungi isolated from leaf litter degrading at low moisture level can be potentially exploited in the form of formulations to accelerate the litter degradation *in situ* in the plantation and in compost pits.

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