

Isolation and Characterization of Indigenous Microorganism (Imo) from Ifugao Bamboo (*Phyllostachys Aurea*) Forest

Chiemela F. Anyanwu¹, Serafin L. Ngohayon², Ricardo L. Idefonso³, Joseph L. Ngohayon⁴

^{1,2} Ifugao State University, Nayon, Lamut, Ifugao, Philippines

^{2,3}Ifugao State University, Potia campus Alfonso Lista, Ifugao, Philippines

Abstract: *This investigation was conducted to isolate and characterized samples of indigenous microorganism (IMO) collected from bamboo forest in Ifugao province Philippines. Plate count was used to determine the relative proportion of different types of microbes in the sample, either as bacteria, fungi or yeast. The samples were serially diluted and the dilutions (10^{-1} - 10^{-8}) were plated on selective media. Microbial isolates showed high bacterial population compared to normal soil population. Eight (8) bacterial isolates as pure culture were relatively the dominant microbes with population ranging from (1.4×10^9 to 5.5×10^8 CFU/g). Bacterial and fungal isolates exhibited distinctive cultural characteristics (color, shape, colony size (mm), texture, elevation, appearance, optical property). Based on the findings of this present investigation, the authors concludes that useful potential bacteria and fungi as indigenous microorganism can be collected from bamboo forest in Alfonso Lista, Ifugao Philippines which can be isolated and used for further studies on bioconversion of farm waste into organic fertilizer.*

Keywords: Microorganism, IMO, bamboo, cultural characteristics, culture media.

1. Introduction

Topsoil contains various amounts of microorganisms. These includes; fungi, algae, bacteria, actinomycetes, and protozoa [1]. These microbes are responsible for the degradation of organic matter. Among these, bacteria and fungi play a major role in the decomposition processes [2].

Microorganisms inhabit almost everywhere on earth where there is moisture and water and play a major role in nutrient recycling in ecosystems acting as decomposers [3]. Using a broad range of enzyme, such as phenol oxidase and cellobiohydrolase, they chemically break down a variety of organic materials. Some microbes fix nitrogen and form an important part of the nitrogen cycle [4].

Indigenous microorganisms (IMO's) are organisms that enrich the soil by speeding up decomposition of organic matter, enabling the release of nutrients. A report on the concept of effective microorganism by Dr. Teru Higa of the University of the Ryukyus in Okinawa Japan, stated that a combination of different microorganism is capable of influencing decomposition of organic matter yielding a life promoting process [5]. Higa, further proposed a dominance principle for effective microorganism, stating that there are three groups of microorganism such as; positive microorganism (regeneration), negative microorganisms (decomposition, degeneration), and opportunist microorganisms that follow the trend of regeneration or degeneration.

The use of microorganism in agriculture has been identified as one major factor that can facilitate and accelerate achievements in a sustainable agricultural program [6]. Chemical fertilizer has been instrumental to the increase in crop yield and the achievement of self-sufficiency in

agriculture over the years. However, it has also caused a lot of pollution in the environment and has led to loss of soil fertility [7]. Residues from agricultural chemicals found in foods have been attributed to the increase in human and animal health hazards. Such problems in agriculture can be alleviated by employing green technology with the aim of fostering sustainable agriculture [8].

Studies have shown that IMO's are able to support number of benefits in agriculture and environment, these includes; organic decomposition, plant nutrition, improvement of crop yield, pest management, disease resistance, tolerance to adverse soil and climatic conditions. Microorganisms has also been used for bioremediation which is considered a more economical and safe method for the treatment of hazardous waste. This involves the use of aerobic as well as anaerobic bacteria for degradation processes for contaminants of soil and water. Fungi specifically, affect soil fertility; controls plant diseases and promote mushroom growth. They also can be used for degradation of plastics [9] and [10].

Indigenous microorganism has also been employed in the technologies of bio-stimulation and bio-augmentation. Bio-stimulation refers to the addition of electron acceptors, electron donors, or nutrients to stimulate naturally occurring microbial populations for the enhancement of bio-remediation processes. Bio-stimulation concept is geared towards boosting the intrinsic degradation potential of a polluted matrix through the accumulation of amendments, nutrients, or other limiting factors and has been used for a wide variety of xenobiotics [12]. Consequently, bio-augmentation refers to the introduction of indigenous microorganism that enhances the biodegradation of chemical compounds that serves as donors of the catabolic genes.

Various microbes can be used to determine biological availability of chemical compound in soil. This is done using bacteria plasmids through endogenous or exogenous procedures which will give indicators of contaminants existing in the environment [13]. Using endogenous procedures, plasmids are isolated from soil bacteria and plated in agar plates which will later be visualized on agarose gels [14]. In a microbial community, fungi play a vital role in biodegradation and conversion processes. It is estimated that the biomass ratio of fungi to prokaryotes in compost is 2:1 [15]. By utilizing carbon source such as lignocellulosic polymers that can survive extreme environmental conditions enabling them to have the ability to sustain compost maturation [16].

Isolation and characterization of microorganisms are important steps in microbes identification and usage. Basic isolation procedures for indigenous microorganism has been suggested by authors. These includes; 1. colony section and purification, 2. selective media and incubation conditions, 3. enrichment and pretreatment of samples, 4. selection of material containing microorganism [17].

Any employed procedure must be geared towards selection of actively growing indigenous microorganism such as bacteria and fungi that constitutes key agents in plant litter decomposition in ecosystems [18]. Furthermore, characterization of indigenous microorganism is an important aspect in the study of microbes. Thus, basic criteria include; morphological and physiological characterization. A better understanding of indigenous microbe's diversity in bioconversion may prove crucial in predicting its application and usage in formulating inoculates.

This work focuses on collection source, isolation and cultural characteristics of indigenous microorganism from a bamboo forest in Alfonso Lista, Ifugao, Philippines which will further elucidate information's for identification studies of potential indigenous microbes for utilization in the decomposition of farm waste for organic fertilizer production.

2. Materials and Methods

2.1 Collection of IMO Samples

Samples of indigenous micro-organisms (IMO) were collected from a bamboo forest in Potia, Alfonso Lista, Ifugao Philippines (Figure 1). Four wooden boxes measuring (12 x 12') were filled with 2kg of steamed rice each and covered with paper towel and plastics. The wooden boxes were buried under a bamboo tree and covered with bamboo leaves for five (5) days.



Figure 1: Collection site of IMO samples



Figure 2 IMO samples

2.2 Isolation of IMO samples and culture by plate count method

Plate count was used to determine the relative proportion of different types of microbes in the sample, either as bacteria, fungi or yeast. The samples were serially diluted and the dilutions (10^{-1} - 10^{-8}) were plated on selective media.

The serial dilutions were setup and one (1) gram of the sample was spread-plated on tryptone glucose yeast extract agar + Cycloheximide (TGYEA) [Ingredients: casein enzymatic hydrolysate, 5 g/l; yeast extract, 3 g/l; glucose, 1 g/l; agar, 15 g/l] for the isolation of bacteria. Yeast malt extract agar + Rose Bengal (YMA) [Ingredients: % malt extract 0.5, yeast extract 0.5, glucose 0.5, agar 1.5] was used for isolation of yeasts and acidified potato dextrose agar (PDA) for isolation of filamentous fungi. The TGYEA plates were incubated at 30 degrees Celsius for 24 hours and the YMA and plates at 30 degrees Celsius for 48 hours, while the PDA plates were incubated at 30 degrees Celsius for 7 days. All plates were incubated under aerobic conditions [19].

Mixed cultures obtained after incubation were named as; IM-Bacteria-1, IM-Bacteria-2, IM-Bacteria-3, IM-Bacteria-4, IM-Bacteria-5, IM-Bacteria-6, IM-Bacteria-7, IM-Bacteria-8 and IM-Fungi-1, IM-Fungi-2, IM-Fungi-3. The number and type of colony forming units (CFU) was evaluated and representative samples were purified by quadrant streaking on sterile NA plates. The purity of cultures was cross checked by gram staining procedure. The viable titer was

then calculated and a circle made at the back of each plate and a number was assigned.

2.3 Total microbial count and morphological characteristics

After successful growth of microorganisms, the colonies were counted using a colony counter (Yc-2A, Prma optical works Ltd, Japan) and colony forming unit (CFU/g) per dilution count were recorded. Mixed cultures of microorganisms named as; IM-Bacteria-1, IM-Bacteria-2, IM-Bacteria-3, IM-Bacteria-4, IM-Bacteria-5, IM-Bacteria-6, IM-Bacteria-7, IM-Bacteria-8 and IM-Fungi-1, IM-Fungi-2, IM-Fungi-3 were characterized for their physical morphology such; color, size, shape. Gram stain was performed to observe the cellular morphology and gram reaction of bacteria.

3. Result and Discussion

3.1 Culture media

In this investigation, culture media used such as; potato dextrose agar (PDA), tryptone glucose yeast extract agar + Cycloheximide (TGYEA) and yeast malt extract agar + Rose Bengal (YMA) and incubated for 24 hours, 48 hours and 7 days at 30⁰ C sustained the growth of microorganism (bacteria, yeast and fungi). Observations revealed that tryptone glucose yeast extract agar + Cycloheximide (TGYEA) was suitable for massive growth of bacteria. Potato dextrose agar (PDA) also proved successful for the growth of fungi while yeast malt extract agar + Rose Bengal (YMA) did not establish significant growth of yeast (table 1). Various nutrient media has been used for the growth and isolation of microorganism. As stated by [20], a suitable nutrient medium for plate count must have basic requirements such as; (1) Standardized composition that can effectively reproduce sufficient accuracy anywhere at any time. In this study, all the selective media used met standardized composition. (2) The media must permit the development of a large range of microorganism present. TGYEA and PDA used in this study established substantial growth rate of microorganisms. Similar growth rates using TGYEA and PDA have also been observed by other authors [21] and [22].

It has been noted that these selective media's (TGYEA, PDA and YMA) are among the most commonly used media for culture and isolation of microorganisms because of their simple formulation and ability to support growth. Several authors [23]-[24]-[25] have reported successful isolation of microorganism exhibiting distinctive growth and development using similar selective media's. Efficiency of TGYEA, PDA and YMA has also been reported by [26]. In this study, the use of PDA supported high population of fungi isolates (4.2 x 10⁴). Similar result was also reported by [27] using solid agar-based media for identifying colony characteristics (shape, size, elevation, margin type) of bacteria, as well as in selecting for particular bacterial groups.

3.2 Total microbial plate count

Bacterial population was highest at 2.8 x 10⁹, followed by the fungi at 4.2 x 10⁴. Yeast population did not fall within the set countable range (table 1). These results indicate that the soil from the bamboo forest where the samples were collected is rich in indigenous microorganism.

Growth of microorganisms depends on various factors such as; source, temperature, pH, incubation period, carbon, moisture, etc. Soil with high moisture content are closely associated with high population of microorganism especially bacteria [28] and [29]. Bamboo forest is predominantly a high moisture environment that has the capacity to influence growth of microorganisms. Growth of microorganism in such environment can be attributed to the high content of organic wastes which provides adequate nutrients (carbon) necessary for microorganisms to carry out decomposition efficiently.

In soils with high amount of organic waste, bacteria populations are usually the most numerous, they make up to 80 to 90% of the billions of microorganisms typically found in a gram of compost [30], and are responsible for most of the decomposition and heat generation in compost. By using a broad range of enzymes, they chemically break down a variety of organic materials [31]. In this study, higher population of microorganism, especially bacteria can be attributed to the abundance of carbon sources from the bamboo tree leaves. In a study by [32] and [33], they reported that an increase in soil microbial population was observed with discharge of effluents from cotton ginning mill and supplementation of animal manure and soil organic matter.

Table 1: Microbial population in the IMO samples

Culture Medium	Incubation period	Type of organism	IMO sample CFU/g
Tryptone Glucose Yeast Extract Agar + Cycloheximide (TGYEA)	30 ⁰ C for 24 hours under aerobic conditions.	Bacteria	2.8 x 10 ⁹
Potato Dextrose Agar + Tartaric Acid (PDA)	30 ⁰ C for 7 days under aerobic conditions.	Fungi	4.2 x 10 ⁴
Yeast Malt Extract Agar + Rose Bengal (YMA)	30 ⁰ C 48 hours	Yeast	<100est *

*IMO sample population measured according to colony forming units C.F. U/g of soil *est. – colony counts did not fall within the set countable range for bacteria and yeasts (25–250) and molds (15– 150).*

Correlation of soil microbial biomass and soil microbial activities are factors showing an indication of soil fertility, hence, mineralization of soil substance, nutrient transformation and microbial population are turn overs that affect the soil fertility [34].

3.3 Morphological characteristics of microbial isolates

Eight (8) bacterial isolates as pure culture were isolated and characterized (Table 2). These 8 bacterial isolates were relatively the dominant bacteria in the sample with population ranging from 1.4×10^9 to 5.5×10^8 . Majority of the bacteria are colored cream to off-cream and opaque except for IM bacteria-3 which has a red center.

Colony size of these bacterial isolates ranged from 1 - 5mm in diameter. Six bacterial isolate had flat elevation and two isolates had convex elevation. Most of the isolates had shiny appearances except bacteria 4 that exhibited dull appearance. Three (3) isolates exhibited opaque optical property while five (5) isolate were translucent. Three fungi isolates were also characterized. Out of the three isolates, one isolate had white cottony with basal and aerial hyphae; no exudates; no soluble pigments; color ranged from smooth cream to yellowish reverse; with colony diameter less than 90 mm. Two isolates had white to yellowish cottony with velvety mycelia; no exudates; no soluble pigment were detected.

Cultural characteristics of microorganism are important for proper identification of microbes. It serves as an adaptation for biological functions geared towards survival and coping mechanisms of microbes when exposed to varying and different environment [35], [36]. In the identification of bacteria and fungi much emphasizes is placed on how the organism grows and performs on media. Thus, cultural characteristics of microbes such as colony morphology can provide useful information concerning motility, pigmentation and oxygen requirements.

While there is variation even among individual strains of the same species of microbes, some characteristics are distinctive, thus can aid in the beginning steps of identification [37] and [38]. Bacteria and fungi isolate in this

study have characteristic growth patterns that can aid in further species identification studies. Differences in the colony morphology, however, can be attributed to the influence of the media and other growth conditions

4. Conclusion

Our findings in this study showed that there is quite a reasonable population of bacteria and fungi in the indigenous microorganism (IMO) sample collected from bamboo forest. This is an indication that these IMO samples can be used for further identification investigation for developing bio-activator for organic waste decomposition. In the choice of media for isolation, the three selective media used (TGYEA, PDA and YMA) were able to initiate growth of bacteria and fungi that produce visible cultural and morphological characteristics, consequently, plate counting which is a well-established method of qualitative evaluation of microorganism proved a reliable method which gave viable populations of bacteria and fungi.

Based on the findings of this present investigation, the authors conclude that useful potential bacteria and fungi as indigenous microorganism can be collected from bamboo forest in Alfonso Lista, Ifugao Philippines which can be isolated and used for further studies on bioconversion of farm waste into organic fertilizer.

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Table 2: Morphological characteristics of microbial isolates from Indigenous microorganisms (IMO) after incubation at 300C

Microorganism/ Isolate Code	Media type	Cultural characteristics						
		Color	Shape	Colony Size (mm)	Texture	Elevation	Appearance	Optical property
IM Bacteria-1	TGYEA	cream	Circular	2-2.5	smooth	flat	shiny	Opaque
IM Bacteria-2	TGYEA	Cream	Circular	1.55	smooth	flat	shiny	Translucent
IM Bacteria-3	TGYEA	red	Circular	5	smooth	flat	Red	Translucent
IM Bacteria-4	TGYEA	off-white	Circular	5	smooth	flat	dull	opaque
IM Bacteria-5	TGYEA	Cream	Circular	4	smooth	flat	shiny	Translucent
IM Bacteria-6	TGYEA	cream	Circular	5	smooth	convex	shiny	Translucent
IM Bacteria-7	TGYEA	cream	Circular	5	smooth	convex	shiny	Translucent
IM Bacteria-8	TGYEA	cream	Circular	5	smooth	flat	shiny	opaque
IM Fungi-1	PDA	smooth cream to yellowish; no exudates; no soluble pigments;	none	>90	White cottony		no soluble pigments	none
IM Fungi-2	PDA	Green powdery basal felt; no exudates; no soluble pigments;	none	35-38	smooth cream to yellowish		no soluble pigments	none
IM Fungi-3	PDA	White to yellowish cottony to velvety mycelia; no exudates; no soluble pigment;	none	25	cream to yellow wrinkled		no soluble pigments	none

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Author Profile



Chiemela F. Anyanwu finished his undergraduate degree in Biology at Philippine Union College, Philippines in 1996. He obtained his MS in Biology at the Institute of Graduate Studies Adventist University of the Philippines in 2000, and PhD in Plant Breeding at the Institute of Graduate Studies, Central Luzon State University, Philippines in 2006. He is presently an Assistant Professor at Ifugao State University (IFSU).



Serafin L. Ngohayon finished his undergraduate degree in Psychology at Isabela State University, Philippines in 1990. He obtained his MA and PhD in Psychology at Hiroshima University, Japan in 1999 and 2002 respectively under the Mombukagakusho Scholarship Program offered by the Japanese Government. He is presently the President of Ifugao State University (IFSU) and President of the International Distance Education Accreditation League (IDEAL).



Ricardo L. Ildefonso finished his undergraduate degree in Agriculture at Nueva Viscaya State Institute of Technology Philippines in 1981. He obtained his MS in Agriculture at Isabela State University in 1987, MA in Technology Education at Central Luzon State University, Philippines in 1992. He finished his PhD at the La Salette University in 1997. Presently, he is the Campus Executive Director of Ifugao State University, (IFSU) Potia campus.



Joseph L. Ngohayon finished his undergraduate degree in Agriculture at Ifugao state college of Agriculture and Forestry, Philippines in 1986. He obtained is MS in crop science at Isabela State University, Philippines in 1990. At present he a Professor at the College of Agriculture and Forestry, Ifugao State University, Potia campus.