

BIBLIOGRAPHY

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ABSTRACT

The study was conducted primarily to identify and assess population of beneficial and pathogenic soil microorganisms in five transitional organic farms and four conventional farms in Benguet, particularly Longlong, Madaymen, Loo, Englandad and Sinipsip.

Four bacterial genera have been isolated and identified from two sampling sites based on their morphological and physiological characteristics. Beneficial bacteria (based on previous works) such as *Pseudomonas sp.* and *Bacillus sp.* have higher population in transitional organic farms than in conventional farms. Conversely, the pathogenic bacterium *Ralstonia sp.* has a higher population on conventional farms when compared to transitional organic farms.

Eight fungal species have been identified based on colony appearance and morphological characteristic of the conidiphore and conidia. Beneficial fungi (based on previous works) such as *Aspergillus niger*, *Trichoderma sp.* and *Gliocladium sp.* have a higher population on organic farms than on conventional farms. On the other hand,

pathogenic fungus such *Fusarium sp.*, has a higher population than on conventional farms.

Application of compost and manures, cover cropping and other practices of organic farmers tend to be related to the increase in population of beneficial microorganisms in the soil.



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INTRODUCTION

It is noted that there is an agricultural intensification over the fast few decades. Twenty-first century farmers become dependent on agrochemicals in stabilizing agricultural production. These chemicals offer effective means in controlling pests, diseases and in the replenishment of soil fertility.

However, agricultural intensification also causes several problems. Enormous application of nitrogenous fertilizers to supply plant nutrition contributed to the problem of soil acidity (Brady and Neil, 1996). This has brought about limited availability of some nutrients and favors the growth of soil-borne pathogens. Moreover, extensive use of chemicals has widespread damaging effects on non-target organisms, the level of species biodiversity and the over all balance and healthy functioning of the environment. Consequently, extensive use of agrochemicals to meet world food population demand significantly altered natural soil microflora.

Several soil microorganisms are known to affect plant growth. Apart from the decomposers of organic materials that released organically held nutrients, others promote plant growth by colonizing root system and increasing the beneficial microbial activity in the rhizosphere. Microbial activity in the soil improves soil structure, increase fixed nitrogen and facilitate nutrient transfer to the higher plants (Tate, 1991). Furthermore, beneficial microorganisms offer biocontrol activity against plant pathogens (Cook, 1991).

Due to the economic and environmental problems of agricultural intensification, the sustainability of both agriculture and the environment is the main objective of today's agricultural policy (Anonymous, 2000). One viable alternative to the more traditional approaches to agriculture is organic farming with the use of biological function and



substitution of chemical fertilizers with farm generated products. This farming system relies in ecologically based practices (Greene and Kremen, 2001) that virtually exclude the use chemicals in stabilizing agricultural production.

Asian governments have recently become interested in organic farming with the expansion of the market for organic products and their potential for promoting sustainable agriculture. Accordingly, almost all have put priority on organic certification and accreditation, even though the major constraints in organic farming in Asia are still at the level of farm production.

The Philippine government has been urged to reintroduce traditional organic methods in crop production in a bid to boost the country's ailing industry. Organic farmer advocate Rivera (2001) insists that organic farming methods could help local farmers address the pest and disease problems currently plaguing the production of the high-value root crop.

In Benguet, 70% of the total vegetable needs of the country produced, farmers continuously rely on using large amounts of synthetic fertilizers and chemical pesticides (Anonymous, 2003). If the trend continues, little if not nil, beneficial microorganisms may permanently be left in the soil. To reverse the trend, viable alternative farming system without harming natural environment and the soil microflora need to be established.

The study provides better understanding and use of beneficial microorganisms to contribute in sustaining agricultural production. Such understanding would make farmers realize the benefits of beneficial microorganisms in crop production. It identifies farm



practices that enhance beneficial microbial activity. Thus, it is important to show and compare microorganisms present in farms practicing conventional and organic farming.

The study was conducted to primarily assess the microorganisms found in both transitional organic and conventional farms. Specifically, it aims to 1) identify beneficial microorganisms (according to Cook and Baker, 1983; Kloepper, 1991) present in the soil under transitional organic and conventional farms; 2) compare for the presence of beneficial and pathogenic microorganisms in transitionally organic farm and conventional farm; and 3) determine cultural practices and organic amendments that enhance the presence of beneficial microorganisms.

Soil samples were obtained from five transitional organic farms and four adjacent conventional vegetable farms within Benguet particularly Loo, Longlong, Englandad, Sinipsip and Madaymen from January to March 2007. Laboratory analysis to determine and identify microorganisms was conducted at the Department of Plant Pathology laboratory.



REVIEW OF LITERATURE

Many microorganisms have enormous diversity of functions in the soil and interact biologically in nature. Beneficial microorganisms play significant roles in the growth and development of plants. In farming, these beneficial microorganisms are fundamental in the mineralization of plant biomass as they improve soil aggregation (Tate, 1991).

Walker (1975) cited that decomposition of organic matter is the primary function of soil microorganism, which releases carbon dioxide, methane, and other volatile compounds. This process releases nutrients, at the same time the glue-like intermediate products and the more resistant portion humus enhanced the stability of soil aggregates.

According to Brady and Neil (1996), microbes convert organically bound forms of Nitrogen, Sulfur, and Phosphorus in plant available forms. However, these soil microorganisms compete in nutrients such as Nitrogen, Phosphorus, Potassium, Calcium and even Iron.

Cook (1991) reported that soil microorganisms offer biological control of soil-borne pathogen. Cook and Baker (1983) define biological control as the reduction of inoculum or disease producing activity of a pathogen in active or dormant state by one or more organism accomplished naturally or through manipulation of natural environment, host or antagonist or by introduction of more antagonists other than man.

Microorganisms that inhabit the soil include bacteria, actinomycetes, fungi and protozoa. Alexander (1977) classified these microorganisms with respect to their carbon and energy sources. Heterotrophic (or chemoorganotrophic), the first classification, require preformed organic nutrients to serve as source of energy and carbon. On the other



hand, autotrophic (or litotrophic) microorganisms obtain their energy from sunlight or by the oxidation of inorganic compounds and their carbon by the assimilation of CO₂. Consequently, fungi, actinomycetes, protozoa, all animals and most bacteria are heterotrophs.

Microbial Community

Several microorganisms are known to affect plant growth. Some beneficial microorganisms isolated in the soil include bacteria, fungi, and actinomycetes.

Bacteria. These comprise a diverse group of single-celled prokaryotic microorganisms, which inhabit the soil of every terrestrial ecosystem (Alexander, 1998). Their density is largely influenced by organic matter content of their habitat, wherein cultivated land is higher than in virgin soil. Bacteria are classified based on their ecological differentiation, physiological differentiation, the ability to grow in the absence of oxygen, and on their cell structure (Alexander, 1961). On the other hand, Bergey's Manual of Determinative Bacteriology classifies bacteria into taxonomic groups based on the classical Linnaean concept of binomial nomenclature (Rao, 1999).

According to Rao (1999), order *Pseudomonadales*, *Eubacteriales* and *Actinomycetales* contain species predominantly encountered in soil. Recent investigation shows that strains of *Pseudomonas*, *Arthrobacter*, *Clostridium*, *Achromobacter*, *Bacillus*, *Micrococcus*, *Flavobacterium*, *Corynebacterium*, *Sarcina* and *Mycobacterium* are the most common soil bacteria.

Foster and Woodruff (1946), as cited by Raymundo *et al.* (1985) characterized genus *Bacillus* as an important antibiotic-producing microorganism that occurs in soil, water and air. The bacterium is spore forming rods, gram positive, motile and aerobic or



facultative anaerobes. Subsequent studies reported successful isolation of antibiotic producing *Bacillus* from the soil (Luke and Conell, 1954, and Tumanyan, 1958) as cited by Raymundo *et al.*, (1985). The bacterium has shown effective control against several pathogens by the production of metabolites with biocontrol and antibiotic properties (Madamba *et al.*, 1991). Raymundo *et al.*, (1985) successfully isolated *Bacillus* species such as *Bacillus cereus*, *B. pumilus*, *B. circulans*, *B. licheniformes*, *B. megaterium* and *B. subtilis* on Luzon area showing antagonism by the production of antibiotics. Mukerji and Garg (1986) showed that *Bacillus subtilis* inhibits germination of *Sclerotium cepivorum*, controls seedling blight caused by *Fusarium roseum* Graminearum and damping off caused by *Pythium ultimum*.

Similarly, Madamba *et al.*, (1991) identified *Bacillus cereus*, *B. stearothermophilus* and *B. circulans* in the Philippine soil reducing root galls of nematode infested plant. They identified *Bacillus cereus* as the most effective in controlling root-knot nematode and even on several pathogens by colonizing root system of the plant. Moreover, *B. cereus* produces probiotics that promote plant growth and induce nodulation of some legume species. Due to these effects, plants grown in *Bacillus* treated nematode infested soil were generally taller and show comparable effects to furadan treatment (Selvadorai *et al.*, 1981) cited by Madamba *et al.*, (1991).

Rhizobacteria, representing fluorescent Pseudomonads, classified by Kloepper and Schroth (1978) cited by Kloepper (1991) as plant promoting bacteria by producing metabolites independent to soil microflora. *Pseudomonas fluorescence* which is Gram negative rod, catalase positive and produces soluble fluorescent pigments improve plant growth of plants by colonizing root region aggressively that preempt the establishment of



Deleterious Rhizosphere Microorganisms (DRMO) on roots (Rao, 1999) and by the production of siderophore pseudobactin that deprives pathogens of iron, thereby permitting the plant to grow better (Cook and Baker, 1983).

A comprehensive study was conducted in IRRI by Rosales *et al.*, (1986) as cited by Davide (1991) on the use of beneficial bacteria for the biological control of plant pathogens. They showed that 50% of paddy field bacterial isolates in the germination test were effective in suppressing bakanae disease caused by *Fusarium moniliforme* on rice. Thus, naturally occurring beneficial bacteria demonstrate potential in controlling bakanae disease (Davide, 1991).

Fungi. These are diverse group of multicellular organisms with incredible array of vegetative and reproductive morphologies (Sylvia *et al.*, 1998). The size, shape and color of conidia or spores and the physiological characteristics of cultures in artificial as well as in natural substrates provide taxonomic criteria in the classification of fungal isolates (Rao, 1999). Fungi inhabit almost any niche containing substrates, take place in degradation of organic matter, agent of disease, beneficial symbionts, agents of soil aggregation, and important food sources for human and many organisms (Sylvia *et al.*, 1998).

The genera of fungi that are most commonly encountered in soils are *Acrostalagmus*, *Botrytis*, *Cephalosporium*, *Gliocladium*, *Monilinia*, *Scopulariopsis*, *Spicaria*, *Trichothecium*, *Verticilium*, *Pullularia*, *Cylindrocarpon*, *Fusarium*, *Absidia*, *Cunninghamella*, *Mortierella*, *Mucor*, *Rhizopus*, and *Pythium*. Germination, root growth and uptake of minerals of plants are enhanced by the synthesis of humic acids by *Alternaria*, *Aspergillus*, *Cladosporium*, *Gliocladium* and *Humicola*. In acidic soils,



Penicillium and *Trichoderma* take part in the cellulose decomposition (Rao, 1999). According to Tancangco *et al.*, (1990), members of genus *Trichoderma* are important sources of commercial cellulose and are natural agents of decomposition of plant material, and have been reported by Cook and Baker (1983) to be very efficient mycoparasite on a wide range of plant pathogens. Alcantara (1978), cited by Davide (1991), observed that *T. glaucum* showed true hyperparasitism on hyphae of *Rhizoctonia solani* and *Trichoderma harzianum* on *Sclerotium rolfsii*. Davide (1991) cited the study conducted by Neypes *et al.*, (1988) proves that the use of *T. glaucum* Abott in controlling foot rot disease of seedling is more effective than fungicides under field condition, and it gives significant increase in yield.

According to Davide (1991), *Gliocladium spp.* has been investigated for their potencial as biocontrol agents against pathogen. Cook and Baker (1983) cited the ideas of Webster and Lonas (1964) that these organisms are common in soil with antagonistic activity against soil-borne diseases by producing potent antibiotics and mycoparasitism. On the other hand, Huang (1978) and Hung Hoes (1976) found out that *Gliocladium catenulatum* kill cells of *Sclerotium sclerotivorum* without direct penetration as cited by (Cook and Baker, 1983).

Aside from the importance of beneficial microorganisms in controlling soil-borne disease causing microorganisms, they also offer biological control on plant parasitic nematodes. Jatala (1981) as cited by Villanueva and Davide (1984) discovered *Paecilomyces lilacinus* fungus in Peru as biocontrol agent against root-knot nematode. The fungus parasitizes egg masses and reduces hatching of larvae of *Meloidogyne incognita* (Reyes and Davide, 1978) cited by Villanueva and Davide (1984).



In the Philippines, Villanueva and Davide (1984) successfully isolated *Paecilomyces lilacinus* Thom and *Arthrobotrys cladodes* Drechs from eggs of root knot nematode infecting tomato, eggplant and in rabbit manure. *P. lilacinus* isolates significantly reduced infection of *Meloidogyne incognita* from 75-82% compared to 90-91 % reduction by the Peru isolate. Davide and Zorilla (1983) also reported that an isolate of *P. lilacinus* significantly control cyst nematode *Globodera rostochiensis* until harvest of potato cv. Isola in Madaymen, Bugias, Benguet. From their experiment, they showed that the effect of the fungus is generally comparable with those treated with nematicides Ethoprop and Carbofuran.

Actinomycetes. These organisms constitute a specialized group of soil bacteria that occur in soils. *Actinomycetes* form aerial mycelium and produce conidia, which give the colonies powdery or chalky appearance (Alexander, 1998) and stick firmly to agar surface (Rao, 1999). In the environment, they are involved in decomposition of organic matter and they are also known as causative agent of animals, plants and human diseases (Alexander, 1998).

The most common genera of *Actinomycetes* found in soil are *Streptomyces*, *Nocardia* and *Micromonospora* (Rao, 1999). Cook and Baker (1983) claim that *Streptomyces* are potentially very effective antagonist of plant pathogenic fungi especially in environment too dry for bacteria by the production of potent antibiotic. In the Peoples Republic of China, Yin and Associates (1957, 1965) cited by Cook and Baker (1983) introduced *Streptomyces sp.* 5406 in cotton at planting and showed improvement in stand and plant vigor.



Natural suppressiveness of soil against soil-borne diseases is directly correlated to soil microorganism, fertility level, and nature of soil and response of growing plants. Adding compost, cover cropping, mulching and manuring foster a more diverse soil environment for myriad soil organisms. Beneficial microorganisms including *Bacillus sp.*, *Pseudomonas spp.*, *Flavobacterium balastinum*, *Streptomyces*, *Penicillium*, *Trichoderma*, and *Gliocladium virens*, suppresses deleterious microorganisms that inhabit compost (Sullivan, 2004).

The aforementioned studies and investigations show that microorganisms are chief factors in sustaining crop production, and suggest that agricultural practices, which significantly affect beneficial microorganisms in the soil, must look into account the viable alternative practices that are ecologically friendly. Therefore, these serve as framework for the analysis in the prevailing study on the assessment of the presence and absence of beneficial microorganisms in conventional and organic farms.



MATERIALS AND METHODS

Soil Sampling

Soil samples were randomly collected from five transitionally organic farms and four adjacent conventional farms in Benguet particularly Loo, Longlong, Englandad, Sinipsip and Madaymen at a depth of 15 cm and mixed evenly. About 1 kg of composite soil samples was placed in a clean plastic for isolation.

Isolation of Soil Microorganisms

Bacteria and Fungi were isolated through the serial dilution method. A 10 gm of soil was diluted with 90 ml of sterile distilled water in a sterile Erlenmeyer flask and mixed using mechanical shaker for 20-30 minutes. While the suspension is in motion, 1 ml of the suspension was withdrawn and added to 9 ml sterile distilled water. Dilutions such as 10^4 for bacteria and 10^2 for fungi were prepared. About 0.1 ml aliquot from each dilution was plated in Nutrient agar (NA) for Bacteria and Potato Dextrose agar (PDA) for fungi, spread using flamed L-shaped glass rod, incubated for 2-3 days and colonies formed were counted. Single colonies that appeared on the medium were transferred on the culture media as mentioned for the pure culture of the isolates.

Characterization of Isolated Microorganisms

Characterization of Isolated Fungi

Isolated fungi were plated on the center of PDA plates, incubated right side up to 30 °C for 7-14 days. Colony color and surface structure were observed.



Wet mount of each isolated fungus was prepared and examined under high power objective. Microscopic structures were observed for presence or absence of septa, color of mycelium, presence or absence of sexual and/or asexual spore, spore type and arrangement of the phialidies.

Characterization of Isolated Bacteria

Morphological and cultural examination. Isolated bacteria were streaked on Nutrient agar (NA), King's Medium B (KMB), which detects formation of water-soluble fluorescent pigments and CPGA+TZC, medium that differentiates bacteria by their ability to convert tetrazolium to a pink to red compound formazan. Different characteristics of bacterial colonies were noted. These characteristics include color, shape, size, elevation and consistency of the colony.

Following Gram staining procedure, the isolated bacteria were classified as Gram-negative rod or coccus or Gram-positive rod or coccus.

Physiological Examination. Physiological characteristics of the isolates were determined following the procedures of Raymundo *et al.*, (1991).

1. Catalase test. A colony of the cultures was transferred on clean glass slide, added with 1-2 drops of freshly prepared 3% hydrogen peroxide (H₂O₂). Bubble formations were observed.
2. Oxidation/Fermentation of carbohydrates and other compounds. Using flamed pin, the cultures were stab in freshly prepared Hugh and Leifson medium. Formation of gas and growth were observed.



3. Gelatin liquefaction test. Cultures were inoculate on tubes of NB +12% gelatin and incubated for 72 hours. Inoculated tubes were placed in ice bath for 15 minutes and observed if the medium remains liquid or it solidifies. Reincubated and observed again after several days.

Other Tests

Motility test. Hanging drop mount of each culture was prepared. A loopful of the culture suspension was placed at the center of a clean cover slip and covered with depression slides centered to the drop of the culture. Quickly and carefully, the cover slip with depression slide was inverted right side up without touching the bottom of the well and observed under low power objective (10x).

Staining Endospores. The prepared smear of the organisms was covered with absorbent paper, flooded with malachite green and steamed for 7 minutes. The slide was thoroughly washed with tap water and counterstained with safranin for 30-60 seconds, washed, blot dried and examined under oil immersion objective.

Determination of Cultural Practices by Organic and Conventional Farmers

Cultural practices in transitional organic and conventional farms were determined by interviewing organic and conventional farmers, following the interview schedule on Appendix A. The information that was obtained is used to relate the presence and absence of beneficial microorganisms in the different farms.



Data Gathered:

1. Colony counts. Microbial population of bacteria and fungi.
 - a. Bacteria. CFU count was obtained using the formula after dilution at 1×10^4 for plating.

$$\text{CFU/ml} = \frac{\text{Average No. of Colonies} / \text{Amount Plated}}{\text{Total Dilution Factor}}$$

- b. Fungi. Colony formed in 1×10^2 fold dilution using the following criteria.

Abundant- average of 8 colonies

Medium – average of 5 colonies

Scarce – average of 2 colonies

Nil- no growth

2. Characteristics of bacterial and fungal isolates

- a. Bacteria

- 1) Morphological characteristics- this includes colony color, shape, consistency and capacity to transmit light.

- 2) Physiological characteristics- this includes catalase test, gelatin liquefaction test and O/F test.

- 3) Biochemical test- this was done through Gram staining.

- b. Fungi

- 1) Colony morphology- this was done mainly through colony color.

- 2) Morphological fungal structures- this was done through microscopic examination.

3. Cultural practices in transitional organic farms. This was done through interview schedule with four transitional organic farmers and five conventional farmers.



RESULTS AND DISCUSSION

Characteristics of the Isolated Bacteria

Four bacterial species were successfully identified in two transitional organic and two conventional farms based on their morphological and physiological characteristics (Figures 1-7). Isolate 1 characterized as gram-negative rod, occurs singly or in chain and produces fluorescent pigment called pyoverdin (formerly called fluorescin) which is a type of siderophore in King's B medium. Cook and Baker (1983) cited that these characteristics are of limited to fluorescent pseudomonad. It grows oxidatively on O/F test producing yellow colony color in open Hugh and Leifson agar tube. The organism was observed to liquefy gelatin, which is one of the distinct characteristic of *Pseudomonas fluorescence* against *Pseudomonas putida*.

Isolate 2 is gram-positive rod, motile and test positive on enzyme catalase. Following endospore staining, the organism appears as rod, and a substantial portion usually contain an oval endospore that makes it bulge. On nutrient agar (NA), colony exhibited large, spreading, and dull to opaque type of colonies with irregular margins. *Bryan et al.*, (1962) identifies this bacterium as to Genus *Bacillus*.

Using CPGA+TZA medium, isolate 3 produces fluidal colonies with light pink to light red centers after 48 hrs. incubation. The bacterium is characterized as gram-negative rod, motile, catalase positive and does not form endospore, which is confirmed by Olson (2005) as *Ralstonia*.

On NA, isolate 4 produces spread mycelia and filamentous gram-positive rod. It is test positive on enzyme catalase and identified as *Streptomyces sp.*





Fig.1. Formation of yellow color on O/F test by *Pseudomonas fluorescens*

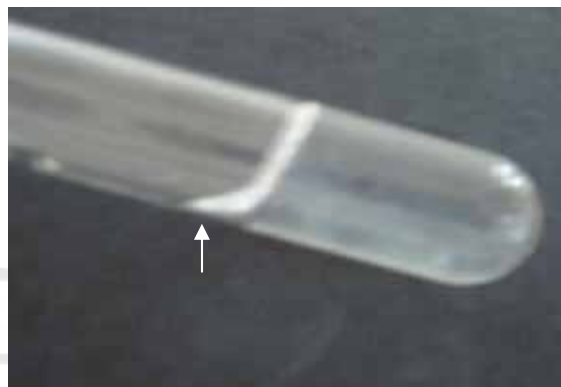


Fig. 2. Gelatin liquefies by *Pseudomonas fluorescens* on gelatin liquefaction test



Fig. 3. *Pseudomonas fluorescens* producing fluorescent green pigment on KMB medium





Fig. 4. Stained oval endospore of *Bacillus* sp. Fig. 5. Spread colony with irregular margins of *Bacillus* sp.



Fig. 6. *Rasltonia* sp. producing light red to pink colony on CPGA+TZA medium

Fig. 7. *Streptomyces* sp. forming spread mycelia



Bacterial Colony Counts in the Soil Samples

Classified beneficial bacteria based on previous studies such as *Pseudomonas fluorescense*, *Bacillus sp.* and *Streptomyces sp.* have higher colony count in transitional organic farms than in conventional farms. Conversely, a pathogenic bacterium such as *Ralstonia sp.* shows higher population in conventional farms than on transitional organic farms (Table 1). Soil microorganisms are known affected by agricultural operations. Nitta (1991) stated that continuous monocropping and application of high doses of chemical fertilizers, soil microflora are easy to become disorder in terms of normal plant growth. Sullivan (2004) cited that adding compost, manure and cover cropping and mulching fosters a more diverse soil environment in which myriad soil microorganisms exist.

Characteristics of the Isolated Fungi

From five transitional organic and four conventional vegetable farms, a total of 8 fungal species were identified (Figures 8-23). Table 2 summarizes the characteristics of the isolated fungi.

Isolate 1 shows a dark green to yellow color in the middle reducing to yellow green as it goes to the edge. Conidia forms in long chain from brushe-shape conidiophores, glubose to ovoid and produce basipetally, which is characterize by Quimio and Hanlin (1999) as *Penicillium*.

Isolate 2 are homogenously black and form large colony on PDA medium with cream to whitish underside. Conidiophores are hyaline, erect, simple, and thick walled, with foot cell basally, inflated at the apex forming glubose vesicles, bearing conidial heads. Biseriate phialides on pale brown glubose vesicles, which is characterize as *Aspergillus niger*.



Table 1. CFU count (10×4)

	ORGANIC		CONVENTIONAL	
	MADAYMEN	LOO	MADAYMEN	LOO
<i>Pseudomonas sp.</i>	1×10^6	--	--	--
<i>Bacillus sp.</i>	9×10^5	1.3×10^6	5×10^5	--
<i>Streptomyces sp.</i>	5×10^5	1×10^6	6×10^5	5×10^5
<i>Ralstonia sp.</i>	2.7×10^6	1.2×10^6	4.8×10^6	3.2×10^6
-- -nil				

Isolate 3 appears in cottony, white to dull pink surface on culture media with white to yellow underside. Conidiophores are erect bearing on brush-shaped conidial bearing apparatus and forming determinate synemmata, branching biverticulate with phialidic and cylindrical conidiogenous cells which is distinct characteristics of *Gliocladium sp.* (Quimio and Hanlin, 1999).

Isolate 4 is characterized under the genus *Trichoderma sp.* producing pale green and become blue green to yellow green colony color as it grows older forming patches and appears in concentric rings. The phialides are converging and arrange approximately at right angles on the conidiophores (Kifer and Morelet, 2000). Conidiophores are branched and arranged in pyramidal order. Phialides are flask-shape and inflated at the base.

Isolate 5 forms a pink colony surface and dark pink underside. Produces arched phragmoconidia. Macroconidia are phragmosporate arched and microconidia are amerosporate, which are limited characteristics of the Genus *Fusarium* (Kifer and Morelet, 2000).



Table 2. Colony Morphology and Microscopic Structures of Isolated Fungi

FUNGAL ISOLATES	COLONY MORPHOLOGY	MICROSCOPIC STRUCTURE
<i>Penicillium sp.</i>	Greenish in the middle to yellow green as it goes to the edge with cream to brownish underside	Forms chain of conidia from brush-shape phialides
<i>Aspergillus niger</i>	Black surface and whitish to cream color at the back of the plates	Long, smooth, colorless conidiophores, biseriate phialides with round and radiate head
<i>Gliocladium sp.</i>	Cottony, white to dull pink and white to cream underside	Conidiophores erect, borne on brush-shaped conidial bearing apparatus, verticillium-like conidiophores on young cultures
<i>Trichoderma sp.</i>	Whitish in early growth, becoming blue green or yellow green patches and appears in concentric rings as it grows mature, cream to yellowish green underside	Conidiophores hyaline, branched, arranged in pyramidal order, phialides hyaline, flask-shaped and inflated at the base
<i>Fusarium sp.</i>	Having pink surface and brownish pink underside	Macroconidia didymo or phragmosporate erect or curved with asymmetrical foot cell, microconidia amerosporate
<i>Pythium sp.</i>	Spread type colony, surface and back of the culture appears in white color	Mycelium coenoncytic, sporangiophore indeterminate, sporangia glubose
<i>Rhizopus sp.</i>	Raised mycelia from the surface of the medium with grayish surface and underside	Mycelia aerial, stolon and rhizoids present, sporangiophores long and upright, sporangia spherical containing minute aplanospores
<i>Cladosporium sp.</i>	Grayish colony color, slow growing, forming black color underside	Spores occurs in chain arising from dark conidiophores, produces prominent scar



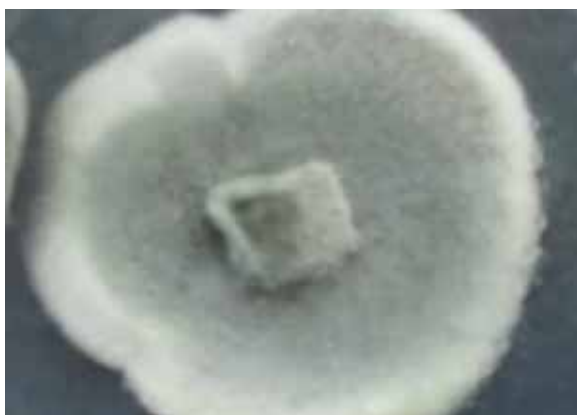


Fig. 8. *Penicillium sp.* with greenish to yellow green mycelia on PDA



Fig. 9. *Penicillium sp.* forming chain of conidia from brush-shaped phialides (400x)

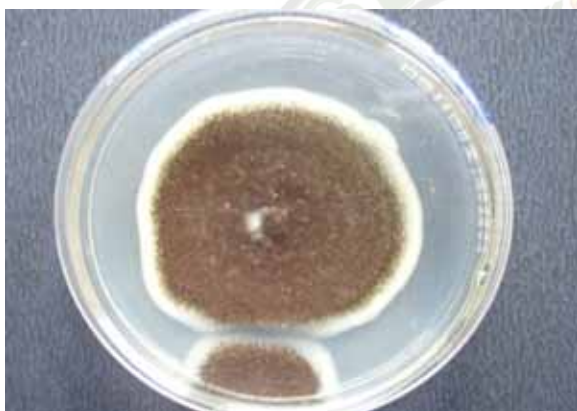


Fig.10. *Aspergillus niger* with homogenous black mycelia

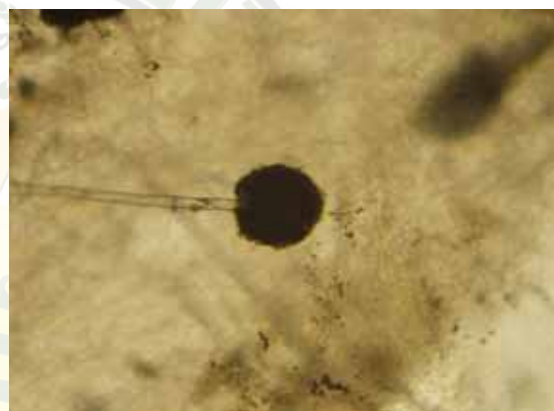


Fig. 11. *A. niger* with biserial phialides with round and radiate head (400x)



Fig. 12. *Gliocladium sp.* showing cottony,

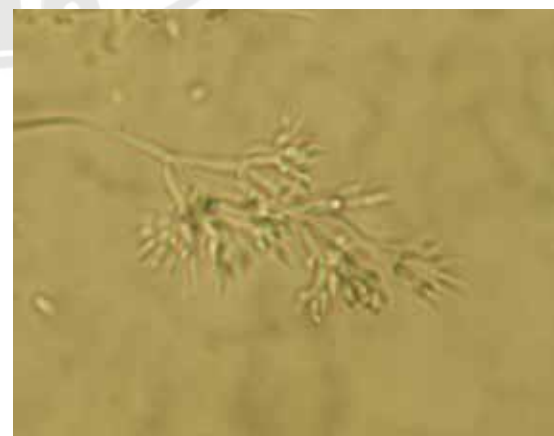
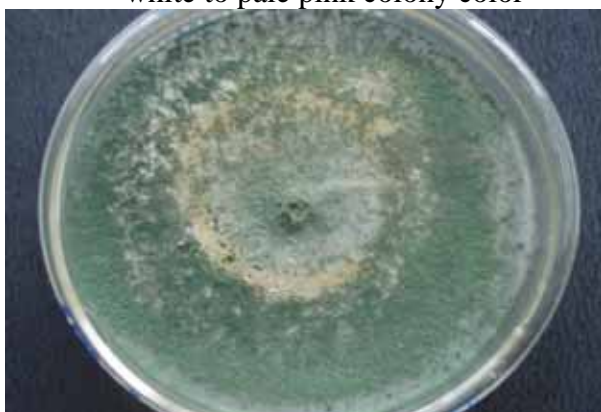


Fig. 13. *Gliocladium sp.* with verticillium-



white to pale pink colony color



like conidiophores (400x)

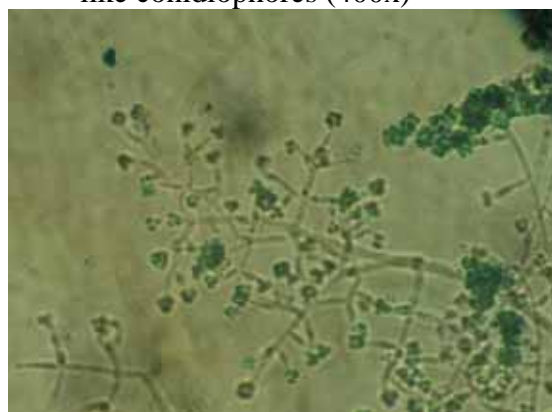


Fig. 14. *Trichoderma sp.* forming green to yellow green colony with concentric rings

Fig. 15. *Trichoderma sp.* with flask-shape and inflated phialides (400x)

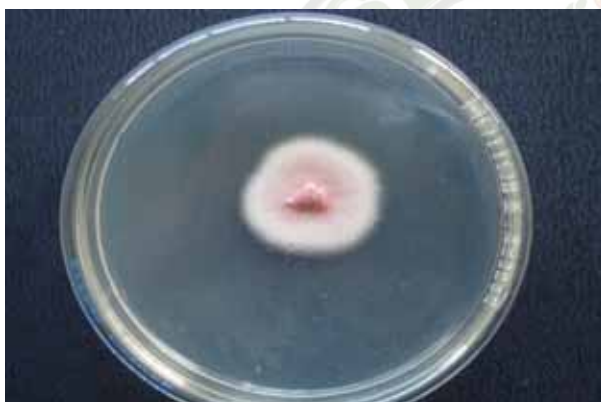


Fig.16. *Fusarium sp.* having pink colony surface

Fig.17. *Fusarium sp.* with arched macroconidia and amero-spore microconidia (400x)

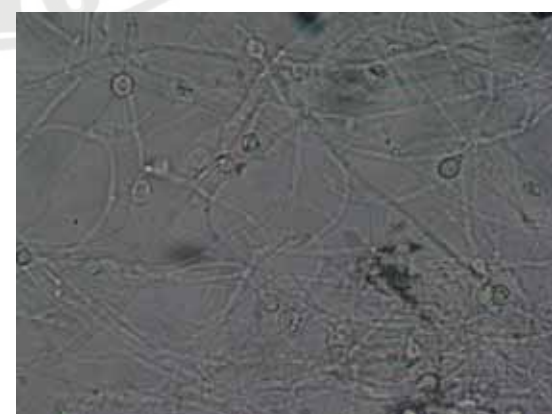


Fig. 18. *Pythium sp.* with white spread type colony

Fig. 19. *Pythium sp.* forming globose sporangia (400x)



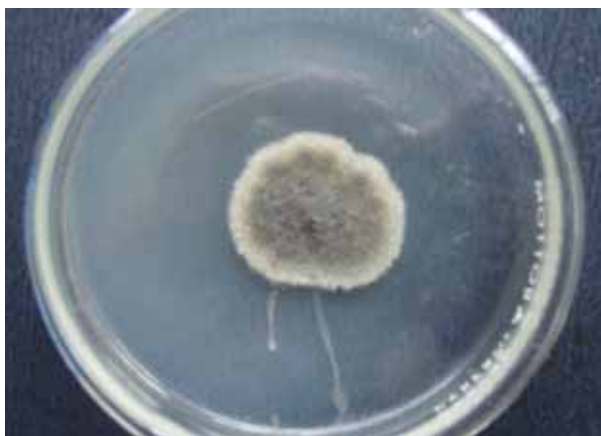


Fig.20. *Rhizopus sp.* with grayish raised mycelia



Fig.21. *Rhizopus sp.* with spherical sporangia containing minute aplanospores (400x)



Fig. 22. *Cladosporium sp.* showing brown to grayish colony, forming lines from the middle extending to the edge

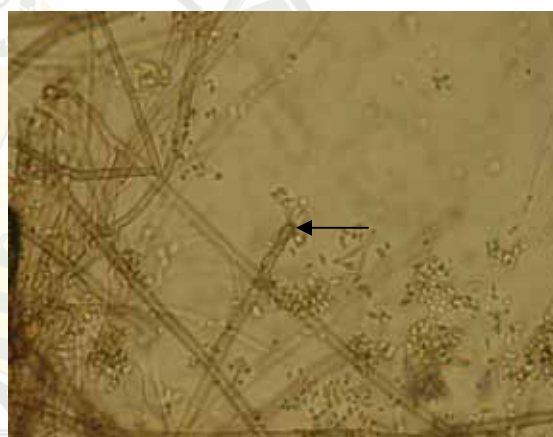


Fig. 23. *Cladosporium sp.* showing a prominent scar (400x)

Isolate 6 forms spread type white colony on PDA. Mycelium appears as coenocytic and sporangiophores are indeterminate with globuse sporangia, which are typical characteristics of *Pythium sp.*



Isolate 7 shows a raised mycelia with dark to white color and yellow underside which is known to be as *Rhizopus sp.* Sporangiohores are upright and long, sporangia appears to be spherical containing minute aplanospores.

Isolate 8 is slow growing, forming dark green to grayish colony color. Spores occurs in chain arise from dark conidiophore producing prominent scar. Conidiophores are macronematous, polyblastic conidiogenous cells usually discrete that is known to belong on Genus *Cladosporium sp.* as stated by Quimio and Hanlin (1999).

Population of the Isolated Fungi

Similar to the bacteria, the beneficial fungi (based on previous works), which include *Trichoderma sp.*, *Gliocladium sp.* and *Aspergillus niger*, have a higher population on transitional organic farms as compared to conventional farms (Table 3). Conversely, pathogenic fungi such as *Fusarium sp.* have a lower population on transitional organic farm than on conventional farms surveyed. However, fungal species such as *Pythium sp.*, *Rhizopus sp.*, *Cladosporium sp.* and *Penicillium sp.*, were observe to have almost the same population in transitional and conventional farms. As stated by Nitta (1991), agricultural practices such as application of high doses of chemical fertilizers, soil microflora are easy to become disorder in terms of normal plant growth. Applying compost, manures, and cover cropping and mulching that adds organic matters to the soil fosters a more diverse soil environment in which a myriad soil organisms exist (Sullivan, 2004).



Table 3. Colonies of isolated fungi on transitional organic and conventional farms

FUNGAL GENERA	COLONY COUNT	
	ORGANIC FARM	CONVENTIONAL FARM
<i>Penicillium sp</i>	8	8
<i>Aspergillus niger</i>	8	2
<i>Trichoderma sp</i>	5	2
<i>Cladosporium sp.</i>	5	5
<i>Gliocladium sp.</i>	2	*
<i>Pythium sp.</i>	2	2
<i>Fusarium sp.</i>	2	5
<i>Rhizopus sp.</i>	2	2

Legend:

* - nil

Organic Farmers Practices

All transitional organic farmers interviewed maintain the fertility level of their farms by applying compost, chicken dung, organic fertilizers and do mulching and other practices that add organic matter to the soil. Some of the farmers introduced beneficial or effective microorganisms available in the market such as *Trichoderma sp.* and *Virtouso*, which contain *Bacillus sp.* According to the farmers, they use *Trichoderma* to control



soil-borne diseases and as plant growth promoter. For controlling pest and diseases, organic farmers use resistant varieties, botanical pesticides and biofungicides such as *Virtouso*.

These practices may explain the presence or absence as well as the population of beneficial fungi and bacteria as earlier presented in Table 1 and Table 3. In a nutshell, transitional organic farm have a higher population of beneficial microorganisms than in conventional farms.



SUMMARY, CONCLUSION AND RECCOMENDATIONS

Summary

The microorganisms found in transitional organic farms and conventional farms are *Bacillus sp.*, *Streptomyces sp.*, *Ralstonia sp.*, *Penicillium sp.*, *Trichoderma sp.*, *Gliocladium sp.*, *Fusarium sp.*, *Cladosporium sp.*, *Pythium sp.*, *Rhizopus sp.*, *Aspergillus niger* and *Pseudomonas fluorescense*, which is only found on transitional organic farm.

Of these microorganisms, those classified beneficial based on previous works include *P. fluorescense*, *Bacillus sp.*, *Trichoderma sp.*, *Gliocladium sp.*, and *A. niger*. These beneficial microorganisms have greater population in transitional organic farms than in conventional farms. Conversely, pathogenic microorganisms, which include *Ralstonia sp.* and *Fusarium sp.* have a greater population on conventional farms than in transitional organic farms. Microorganisms such as *Pythium sp.*, *Penicillium sp.*, *Rhizopus sp.* and *Cladosporium sp.* have the same population on both transitional and organic farms.

Conclusion

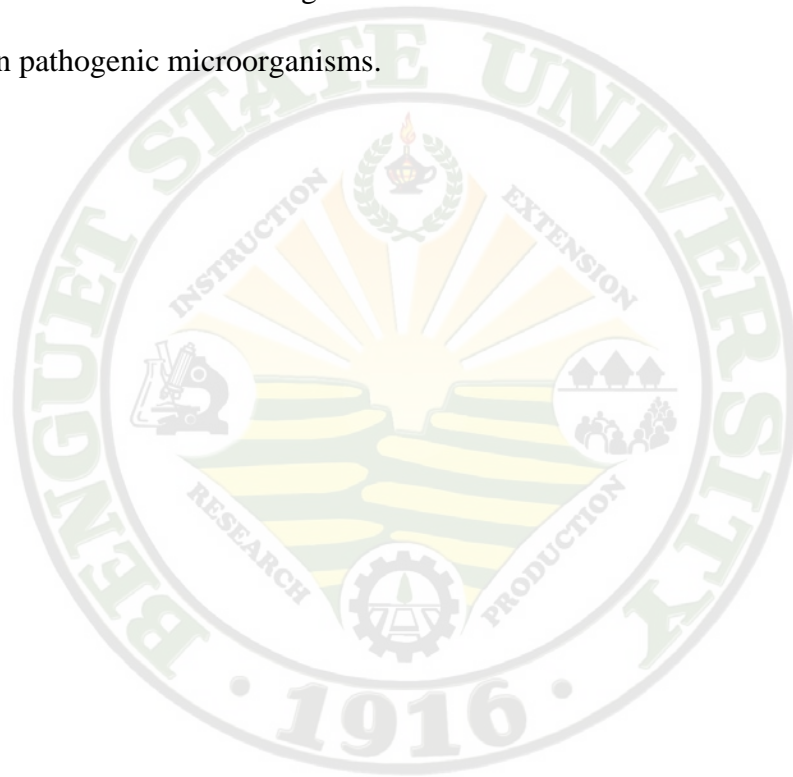
There are beneficial microorganisms (based on previous works) present in transitional organic farms in Benguet. Said beneficial microorganisms have higher population in transitional organic farms than in conventional farms. Conversely, some pathogenic microorganisms have a higher population in conventional farms.



Recommendations

Based from the results of the study, the following are suggested.

1. Pathogenicity test need to be conducted among the isolates to determine their efficacy against certain pathogenic species.
2. Further characterization of the isolates including DNA analysis need to be undertaken.
3. There is a need to investigate the mode of action of beneficial microorganisms on pathogenic microorganisms.



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APPENDIX

Appendix A. Survey Questionnaire

I. Personal information

1. Name: _____
2. Sex: _____
3. Address: _____
Farm Location: _____
4. Educational attainment
 a. no formal education g.college graduate (degree)
 b. elementary level _____
 c. elementary graduate h. vocational/ diploma
 d. high school level _____
 e. high school graduate
 f. college level
5. Trainings and seminars attended:

II. Farm Management

1. How many years have you been in farming? _____
2. How many years have you been farming organically? _____
Conventionally? _____
3. Is your farm certified? Yes No
4. Is all your production organic/conventionally produce? Yes No
Or do you mixed organic and conventional farm? Yes no
5. Do you farm full time or part time? _____
6. What is the total area devoted to organic farming? _____
On vegetable organic farming? _____
7. Crops grown: _____
Estimated area: _____
8. Cropping pattern
 Monocropping: _____
 Mixed cropping: _____
 Crop rotation: _____



III. Production Management

1. Tillage operation

- Cultivate land using \uparrow grabhoe \uparrow machines
 \uparrow others _____
 No tillage

2. Pest and Disease Management

a. Weeds: _____

Management measures

- Mechanical tillage
 Weeding by hand
 Cover crops: _____
 Mulches: _____
 Burning: _____
 Chemicals: _____
 Other control: _____

b. Insects: _____

Management measures

- Use of resistant varieties: _____
 Crop rotation: _____
 Mixed cropping: _____
 Beneficial insects: _____
 Botanical pesticides: _____
 Chemical pesticides: _____
 Use of traps \uparrow plants \uparrow pheromones
 \uparrow others (specify) _____

c. Diseases: _____

Management measures

- Use of resistant varieties: _____
 Crop rotation: _____
 Mixed cropping with: _____
 Beneficial microorganisms: _____
 Botanical pesticides: _____
 Chemical pesticides: _____
 Other control: _____

3. Fertility management

- | | Materials used |
|--|----------------|
| <input type="checkbox"/> Compost application | _____ |
| <input type="checkbox"/> Green manuring | _____ |
| <input type="checkbox"/> Organic fertilizers | _____ |



___ Mulching	_____
___ Cover cropping	_____
___ Fermented products	_____
___ Effective microorganisms	_____
___ Balancing soil pH	_____
___ Synthetic fertilizers	_____
___ Others (Specify)	_____
_____	_____

