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ISOLATION, PURIFICATION AND IDENTIFICATION OF ANTIBIOTIC PRODUCING ACTINOMYCETES ISOLATED FROM COMPOST PREPARED FOR MUSHROOM GROWTH

[1]Mulatu Workie*, [2]Dawit Abate

[1]*Ethiopian Institute of Agricultural Research* (EIAR)*, Holeta National Agricultural Biotechnology Research Center- Microbial Biotechnology Program,* ***ETHIOPIA***

[2]*Assistant Professor at Addis Ababa University Microbial Cellular and Molecular Biology Department,* ***ETHIOPIA***

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Corresponding Author:
Mulatu Workie
Ethiopian Institute of Agricultural Research (EIAR),
Holeta National Agricultural Biotechnology Research Center,
ETHIOPIA
Email: *mulatudst6@gmail.com*
Phone: *+251910030448*

Abstract

Actinomycetes are well adapted to growth on solid substrates and are ubiquitous in natural environments because of their metabolic diversity and evolution of specific mechanisms of dispersal. Thus, the present study has been designed to isolate, purify and identify antibiotic producing actinomycete isolates from compost prepared for mushroom growth. A total of 28 actinomycetes were isolated from mushroom compost. In the present study, the compost sample analysis results for temperature, pH, moisture content and carbon nitrogen ratio were varied with respect to sampling days. The average temperatures attained in sampling days 7,13,19,25 and 31 were 42.67±3.78, 62.67±2.51, 38.00±1.00, 41.00±2.64 and 38.67±1.52 degrees celsius respectively. The pH varied with time during the composting process. Similarly, the average moisture contents of the compost sampling days, 7,13,19,25 and 31 were 25.76±0.49, 41.13±0.40, 33.28±0.37, and 44.07±0.17and 32.21±0.22 percent, respectively. At the beginning of sampling the C: N ratios of sampling day 7 were 25.59±0.53 and at the end of composting C:N ratio was 12.17±0.18. All the isolated actinomycetes were grow on different growth medium with some variation by colour. Most of the isolates were efficient in hydrolyzing starch except a few strains, but catalase was positive in all the isolates. Based on their morphological, cultural and biochemical characterization four isolates were identified to the genus Streptomyces, two were Sacharomonoospora. The finding of the current study showed that mushroom compost is rich with antibiotic producing actinomycetes so, the diversity of the mesophilic and thermophilic actinomycetes in mushroom compost could be further studied.

Key words
Actinomycetes, Characterization, Mushroom Compost.

INTRODUCTION

The name 'Actinomycetes' was derived from Greek 'akitino' (a ray) and 'mykes' (fungus) and given to these organisms from initial observation of their morphology. They are diverse group of gram positive bacteria that usually grow by filament formation and have high G+C (>55%) content in their DNA. Actinomycetes has been classified to the order Actinomycetales (Superkingdom: Bacteria, Phylum:Firmicutes, Class: Actinobacteria, Subclass: Actinobacteridae) **[25]**. They are free living, saprophytic bacteria widely distributed in soil, water and colonizing plants showing marked chemical and morphological diversity but form a distinct evolutionary line of organisms **[12]**. Actinomycetes are well adapted to growth on solid substrates and are ubiquitous in natural environments because of their metabolic diversity and evolution of specific mechanisms of dispersal. There are many environments that reach

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temperatures of 40-70°c; these include solar heated soil and decaying plant matter of which composting is a good example. The material being composted provides a source of nutrients, moisture and microbial inoculum. Preparation of mushroom compost is a good example of commercial process in which thermophilic bacteria play key role. It is an aerobic, solid substrate (chicken manure) fermentation mediated by a diverse, active, highly competative, largely gram positive bacterial microflora of which the thermophilic actinomycetes comprise an important component **[7]**. (Composting is the biological conversion of solid organic material into usable end products such as substrates for mushroom production, or biogas (methane). Regardless of the product, the active component mediating the biodegradation and conversion processes during composting is the resident microbial community. Therefore, optimization of compost quality is directly linked to the composition and succession of microbial communities in the composting process. This means that tools are required to monitor and characterize microbial communities during the composting process and to relate microbial communities to compost quality **[26]**. Under suitable conditions, the initial decomposition of these nutrient-rich substrates by mesophilic microbes (including actinomycetes) leads to self-heating, which provides ideal conditions for rapid growth of thermophilic actinomycetes. Thermoactinomyces and Saccharomonospora species are obligate thermophiles; other genera, such as Micropolyspora, Pseudonocardia, Streptomyces, and Thermomonospora, contain species that are either obligate or facultative thermophiles. Such actinomycetes therefore predominate in self-heated substrates **[11]**. The rise in temperature ultimately suppresses their growth and they become superseded by thermophilic bacteria, fungi and actinomycetes. The temperature continues to rise to the peak heat phase (70-80°c), which encourages the proliferation of other thermophiles **[32]**. Many factors determine the microbial community during composting. Under aerobic conditions, temperature is the major factor that determines the types of microorganisms, species diversity and the rate of metabolic activities **[16]**. Thus, the present study has been designed to isolate, purify and characterize antibiotic producing actinomycete isolated from compost prepared for mushroom growth.

MATERIALS AND METHODS

Collection of compost samples

Compost used in this study was a commercial preparation produced from Teff straw, 170kg; horse manure, 80kg; chicken manure,30kg; sawdust, 70kg and gypsum, 25kg **[29]**. The piles were constructed with 1m height, length and width ($1m^3$) above 10 cm height of wooden bed. Traditional scheme of mixing and moistening the ingredients for compost preparation was applied. During composting the pile was turned or the composting materials were mixed every four days interval and compost samples from treatment windrows were collected five times at 7, 13, 19, 25, and 31 days intervals during the composting period. A total of twenty different compost samples from different stages of mushroom compost were taken and actinomycetes were isolated using appropriate medium.

Analysis of Physical and Chemical Properties of the compost

Moisture Content

The moisture content of the compost was determined by the gravimetric method **[9]**. A crucible, dried at 105°C in an oven overnight, was weighed and recorded as Wcrucible. A 5.00 g wet sample was placed in the weighed crucible. The total mass of the crucible and sample material was recorded as Wwet-total. The crucible was placed in an oven for 24 h at 105 ± 5.00°C. After drying, the crucible was cooled to room temperature for 24 h in a desiccator (a glass container containing a drying agent to absorb moisture). The final weight of the cooled crucible was recorded as Wdry-total and the moisture content of the compost material on day i was calculated as:

$$\text{Moisture content i (\%)} = \frac{\text{W wet-total i} - \text{W dry-total i}}{\text{W wet-total i} - \text{Wcrucible i}} \times 100\%$$

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Temperature

Temperature was measured using an electronic digital thermometer for 5 days on the compost site by inserting the thermometer inside the windrow at three different points and the average of three readings was recorded **[21]**.

pH

The pH. A 5.00 g of compost sample was weighed and placed in a 250ml flask containing 25 ml of deionized water. The sample was stirred for 15s to create a slurry solution **[30]** and then left for another 30 min to settle. The pH was read by inserting the electrode into the upper part of the suspension. Carbon to Nitrogen Ratio (C:N)

Total Nitrogen determination

Total nitrogen was determined by the Kjeldhal procedure as described by **[18]**. A 0.3g of dried compost sample was taken in digestion flask, acidify the sample with 10 ml of concentrated sulfuric acid and 3ml of H_2O_2. About 3g of $CuSO_4$, 10g of K_2SO_4 and 0.3g of selenium catalyst mixture was added. The flask was swirled in order to mix the contents thoroughly then placed on heater (370^0C) to start digestion. Then distillation took place by adding 25ml of 40%NaOH and using 25 ml of 2%boric acid and 10 drops of indicator solution. Finally, the distillate was titrated with standardized 0.1N sulfuric acid till the appearance of a reddish color. The total nitrogen percent content was determined using the following formula:

$$\text{Total nitrogen (percent) by weight} = \frac{(a-b) \times N \times 0.014}{S} \times 100\%$$

Where: a: ml of H_2SO_4 required for titration of sample, b: ml of H_2SO_4 required for titration of blank
S: air dry sample weight in grams, N: Normality of H_2SO_4, 0.014 - molecular weight of nitrogen in gram

Organic carbon (OC) determination

Total organic carbon was determined by a procedure described by **[28]**. 0.3g of compost sample was taken into 500 ml Erlenmeyer flask. To this 10ml of 1N $K_2Cr_2O_7$ was added and shaken gently to disperse the compost sample into the solution. Twenty (20ml) of Concentrated H_2SO_4 was added rapidly into the solution, and immediately mix by swirling gently for 30minutes to mix the suspension. Two hundred (200ml) of distilled water was added. Then add 10ml concentrated orthophosphoric acid just before titration and add 0.5ml of barium diphenylamine sulphonate indicator. Finally, titrate with 0.5N ferrous sulphate solution until the color changed from violet blue to green. Sample without soil served as blank. The percent of carbon content was determined using the following formula.

$$\% \text{ of organic carbon} = \frac{(V1 - V2) \times N \times 0.39 \times 100\%}{S}$$

Where: N- Normality of ferrous sulphate solution from blank titration

$$N = \frac{(NK2Cr2O7 \times VK2Cr2O7)}{VFeSO4}$$

V1: ml ferrous sulfate solution used for blank, V2: ml ferrous sulfate solution used for sample,
S: weight of air dry sample in gram, 0.39: equivalent weight of carbon
The carbon: nitrogen ratio of the prepared compost is determined from the quotient of total organic carbon to total nitrogen (Martin, 1991).

$$C: N = \frac{TOC (\%)}{TN (\%)}$$

Where, TOC: Total organic carbon, TN: Total nitrogen

Isolation of actinomycetes from compost

Media preparation for isolation of actinomycetes

Actinomycetes were isolated by spread plate technique following the serial dilution of compost samples on Starch Casein Agar **[33]**. Streptomycin 40µl/ml and griseofulvin 50µl/ml were used to prevent bacterial and fungal contaminants, respectively **[1]**. Ten (10g) of compost sample was taken in a 250 ml Erlenmeyer flask containing 90 ml of sterile water and shaken on a rotary shaker for 1 hour. A serious of culture tubes containing 9ml of sterile water was prepared; 1ml suspension was transferred aseptically to the first tube (10^{-2})

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and mixed well on vortex. Further serial dilutions were made to produce 10^{-3}, 10^{-4}, and 10^{-5} suspensions. According to **[4]** suspension (0.1ml) from 10^{-4} and 10^{-5} culture tubes were spread evenly with L -shaped glass rod over the surface of sterile Starch Casein Agar. The plates were incubated at 25°C for 7 days. The plates were observed intermittently during incubation. The selected isolates were further purified by multiple streaking methods and then stored, at 4°C for further use.

Identification of selected isolates

Based on their antimicrobial properties, isolates were chosen for the further morphological, cultural and biochemical characterization.

Morphological identification of isolates

Morphological methods consisted of macroscopic and microscopic methods. All the isolates were identified up to genus level according to the methods described in the **[5]**. The characteristics were done on the basis of morphology of spore chain, color of aerial mycelium, color of substrate mycelium, gram staining, growth on actinomyces media, etc.

Macroscopic characterization

Cultural characteristics of the isolates were investigated by growing the isolates on, three different growth medium such as Starch Casein Agar, Starch Nitrate Agar, and Chitin agar. A loop full of each isolate from 7 days old culture was taken and inoculated into each of the medium by streak plating technique and incubated at 30°C for 7 days. The experiment was done in duplicates and colony morphology was noted with respect to color of aerial mycelium and substrate mycelium and growth characteristics was examined **[2]** and the result was recorded.

Microscopic characterization

The microscopic examination was carried out by cover slip culture and Gram staining methods to study the morphology of the isolates. Cover slip culture was done by inserting sterile cover slip at an angle of 45°c in Starch Casein Agar Medium. A loop full of actinomycetes isolates were taken from 7 day old culture and inoculated, at the insertion of the sterile cover slip on the medium and the plates were incubated at 28±2°C for 5-7 days. The cover slips were removed from the cultured medium using sterile forceps and placed upward on a glass slide. The growth on the cover slip was fixed with few drops of absolute methanol for 15 minutes, and washed with tap water and flooded with crystal violet reagent for one minute followed by washing and blot drying and observed under the microscope. The morphological features of aerial and substrate mycelium was observed and recorded among the isolates, predominant organisms were selected for further studies **[19]**.

Biochemical characteristics

The isolates were characterized based on hydrolysis of starch, catalase production test, nitrate reduction and gelatin hydrolysis **[37]**.

Test for a mylolytic activity by starch hydrolysis

According to **[15]** the determination of amylase producing microorganisms starch agar media were used. From 7 days old culture were streaked on the pre dried surface of the agar plates. The streaked plates were incubated at 30°c for two days. After incubation starch hydrolysis was determined by the addition of Lugol's iodine solution. Organisms that form colonies surrounding by clear zone were considered as amylase producers as exoenzyme.

Catalase test

Catalase test was carried out after young colonies were flooded with 3% solution of hydrogen peroxide (H_2O_2).The formation of bubbles indicated the presence of catalase **[15]**.

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Gelatin hydrolysis

This test was done on sterile Nutrient Gelatin Media. Nutrient gelatin contains beef extract (3gm/l) and peptone (5gm/l) to support the growth and enough gelatin (120g/l) to cause medium gel. Isolates were inoculated in to nutrient gelatin test tubes with a sterile needle. The tubes were incubated at 35^{0}c for 10 days. The medium chilled thoroughly in to a refrigerator before examination. Chilling is essential because gelatin is liquid at temperature above 20^{0}c **[31]**. The tubes were examined to see whether the medium was liquid for positive test or solid to confirm negative test after end of incubation periods.

Nitrate reduction test

The selected isolates were grown in a nitrate containing broth (Starch Casein Broth).Then inoculated with 7th day old and incubated at 28°C for 7 days. Controls were also run without inoculation. On 7th day, the clear broth was tested for the presence of nitrite. Add two drops of sulphanilic acid solution (8g of sulphanilic acid in 1000 ml of 5N acetic acid) followed by 2 drops of N-N-Dimethyl-α-napthylamine (10g of α-naphthalamine in 1000 ml of 5N acetic acid) solution was added. The presence of nitrite was indicated by a pink, red or orange colour and absence of colour change was considered as nitrite negative. In the later case, the presence or absence of nitrate in the broth under examination was confirmed by adding a pinch of zinc powder after the addition of the reagents; the unreduced nitrate, if present, would give a pink, red or orange color **[3]**.

DATA ANALYSIS

The data were analyzed using descriptive statistics, and the results were presented as mean ± SD. The statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Post Hoc Multiple Comparison Tests using statistical software (SPSS) package version 17.0 for windows and P values < 0.05 were considered as significant.

RESULT

Analysis results of composting raw materials

The analysis of compost samples with regard to temperature, pH, moisture content and carbon nitrogen ratio with respect to sampling days are presented in Table 1 below. The average temperatures attained in sampling days 7,13,19,25 and 31 were 42.67±3.78, 62.67±2.51, 38.00±1.00, 41.00±2.64 and 38.67±1.52 degrees Celsius respectively. The highest temperatures of all compost sampling days were recorded at 13th day of sampling, followed by decrease on the subsequent sampling days. The pH varied with time during the composting process ranging from 8.23 at the 7th day of sampling, with the highest pH record of 8.87 at the 25th day of sampling. Similarly, the average moisture contents of the compost sampling days, 7,13,19,25 and 31 were 5.76±0.49,41.13±0.40,33.28±0.37 and 44.07±0.17 and 32.21±0.22 percent, respectively. At the beginning of sampling the C:N ratios of sampling day 7 were 25.59±0.53 and at the end of composting C:N ratio was 12.17±0.18. The C:N ratio of composting had decreased during the composting period (Table 1).

Table 1: Showing that analysis of physical and chemical properties of mushroom compost

Sampling days	Parameters			
	Temperature (^{o}c)	pH	Moisture content (%)	C:N ratio
7	42.67±3.78^b	8.23±0.03^c	25.76±0.49^e	25.59±0.53^a
13	62.67±2.51^a	8.64±0.11^a	41.13±0.40^b	16.10±0.10^b
19	38.00±1.00^b	8.50±0.17^b	33.28±0.37^c	14.18±0.22^c
25	41.00±2.64^b	8.87±0.15^a	44.07±0.17^a	12.21±0.29^d
31	38.67±1.52^b	8.69±0.04^a	32.21±0.22^d	12.21±0.29^d

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Isolation of Actinomycetes from mushroom compost

In the present study, about twenty eight actinomycetes were isolated from mushroom compost, with the isolation media such as Starch Casein Agar (SCA), Starch Nitrate Agar (SNA) and Glucose Asparagine Agar (GAA). Among which 12 isolates were screened for antifungal activity. Screening of the isolate for antimicrobial activity was made after grouping the isolates in to twelve groups based on morphological similarity. Out of the twelve isolates ten isolates (35.71%) showed antifungal activity against one test fungus in primary screening **[24]**.

Identification of selected actinomycetes isolates

The six actinomycetes isolates, selected through the microbial sensitivity test were taken for morphological, cultural and biochemical characterization.

Morphological characterization

Spore chain morphology

This was done by the cover slip culture technique. The slides were examined under microscope of 100x. Among the isolated actinomycetes strains AAUCS2WB8, AAUCS2WG6, AAUCS2BB12, AAUCS2Y10 and AAUCS2GP11 showed flexible types of spore chains whereas strain AAUCS2PY7 showed spirals spore chains (Table 2).

Table 2: Microscopic observation of selected actinomycetes isolates spore chain morphology

Codes of isolates	AAUCS 2WB8	AAUCS 2WG6	AAUCS 2PY7	AAUCS 2BB12	AAUCS 2Y10	AAUC S2GP11
Spore chain morphology	RF	RF	S	RF	RF	RF

NB: RF = Rectiflexibiles, S = Spirals

Cultural Characteristics

The cultural characteristics of isolates were studied by growing them on different growth medium. All of the isolates grew very well on three different cultural medium such as Starch Casein Agar, Starch Nitrate Agar and Chitin Agar. The growth characteristics, presence of aerial and substrate mycelium were observed (Table 3). The isolate, AAUCS2WG6, AAUCS2PY7, AAUCS2WB8, AUCS2Y10, AAUCS2GP11 and AAUCS2BB12 exhibited excellent growth on Starch Casein Agar medium, Very Good growth was detected on Starch Nitrate Agar Medium and moderate growth was observed on Chitin Agar. The color of aerial and substrate mycelium was varied on the different culture medium used.

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Table 3: Cultural Characteristics of Isolates using different growth media

Codes of Isolates	Growth Medium								
	SCA			SNA			CA		
	Aerial Mycelium	Substrate Mycelium	Growth Aerial	Aerial Mycelium	Substrate Mycelium	Growth Aerial	Aerial Mycelium	Substrate Mycelium	Growth Aerial
AAUCS2 WG6	Whitish grey	Dark Grey	+++	Whitish grey	Dark Grey	++	Whitish grey	Light Grey	+
AAUCS2 PY7	Pale Yellow	Light Orange	+++	Pale Yellow	Light Yellowish	++	Pale Yellow	Light Yellow	+
AAUCS2 WB8	Whitish Black	Dark Brown	+++	Black	Light Black	++	Whitish Black	Dark Black	+
AAUCS2 Y10	Yellow	Orange	+++	Yellow	Yellow	++	Yellow	Yellow	+
AAUCS2 GP11	Grey Pink	Grey	+++	Grey Pink	Grey	++	Grey Pink	Light Grey	+
AAUCS2 BB12	Blue Black	Light Yellowish	+++	Blue Black	Dark Black	++	Blue Black	Dark Black	+

NB: SCA =Starch casein agar, SNA = Starch nitrate agar, CA =chitin agar, +++ = Excellent growth, ++ = Very good growth, + = Good growth

Biochemical characteristics

All the six isolates namely AAUCS2WB8, AAUCS2WG6, AAUCS2PY7, AAUCS2GP11, AAUCS2BB12 and AAUCS2Y10 showed positive results in starch hydrolysis and catalase test except isolate AAUCS2GP11 which showed catalase negative. Isolates, AAUCS2WB8, AAUCS2WG6, AAUCS2BB12 and AAUCS2Y10 showed positive results in gelatin hydrolysis test, whereas AAUCS2PY7 and AAUCS2GP11 were negative. Except isolates AAUCS2Y10 and AAUCS2BB12, all of them showed nitrate reductase activity.

Table 4: Biochemical test of actinomycetes isolates

Codes of isolates	Starch hydrolysis	Gelatin hydrolysis	Catalase	Nitrate reductase
AAUCS2WB8	+	+	+	+
AAUCS2WG6	+	+	+	+
AAUCS2PY7	+	-	+	+
AAUCS2GP11	+	-	-	+
AAUCS2BB12	+	+	+	-
AAUCS2Y10	+	+	+	-

NB: + = Positive, - = Negative

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DISCUSSION

Compost samples used in this study varied greatly in their physical and chemical properties (Table 1). The average highest temperatures attained in 13th sampling days were 62.67±2.51 degrees celsius, where as in the 7, 19, 25 and 31 sampling days the temperatures were 42.67±3.78, 38.00±1.00, and 41.00±2.64 and 38.67±1.52 degree celsius, respectively. The highest temperature degrees of all compost sampling days were recorded at the second turning stage. As it is seen from Table 1, the temperature of the compost piles steadily rose to a peak level at the 2nd sampling days followed by gradual decrease. Increased temperature of the 13th days of composting is an indicator for a rapid and exothermic microbial activity within compost layers. This may be a critical stage for decomposition of carbohydrates **[36]**. The temperature of compost is important in several perspectives. As heat is generated by the oxidation of organic matter it is symptomatic of progress of the process. Microbial processes of thermophiles and chemical reactions are faster at higher temperatures **[27]**. The maximum temperatures of the composting treatment windrows were found to be within the optimum temperature ranges 40-75^{0}C which was reported by **[8]**. In this study, pH values of the compost were at alkali levels ranging from 8.23 at the 7th day of sampling, with the highest pH record of 8.87 at the 25th day of sampling and thus support the growth of bacteria. The pH varied with time during the composting process and is a good indicator of the extent of decomposition within the compost mass. During the composting period the pH was alkaline in all sampling days. The composting process average pH was between 7 and 8.6. Even though, the optimum pH range for most bacteria is between 6.0 and 7.5 the rise in pH does not cease the activity of microorganisms since decomposition can take place up to pH 9 **[13]**. The pH of treatment windrows were almost in alkaline range that might have influenced the quantity of nitrogen presents in the treatment windrows. Windrows that have relatively high pH are found to have lower quantity of total nitrogen. This might be because of the loss of nitrogen through volatilization in the form of ammonia at alkaline condition as reported by **[35]**. The moisture content of the five sampling days showed varying results (Table 1).The moisture content of the compost was monitored periodically during the composting period and the average moisture contents of the compost sampling days,7,13,19,25 and 31 were 25.76±0.49, 41.13±0.40, 33.28±0.37, 44.07±0.17 and 32.21±0.22 percent respectively. At the beginning of sampling the C:N ratios of sampling day 7 were 25.59±0.53 and at the end of composting C:N ratios were 12.17±0.18. The C:N ratios of 19th, 25th and 31th days of sampling had less than 15. Windrows have C:N ratios less than 15 that would not cause nitrogen immobilization instead favors nitrogen mineralization as it is indicated by **[22]**. C:N ratio of composting had decreased during the composting period. The C:N ratio of sampling day 7,13,19,25 and 31 had deceased drastically from 7th to 31th days respectively (Table 1).The C:N ratio is usually employed to indicate the maturity degree of compost **[6]** reported that decrease in C:N ratio implies an increase in the degree of humification of organic matter (Table 1). High C:N ratio indicates the presence of unutilized complex nitrogen; whereas completion of the process (compost maturity) is indicated by the reduction of ratio to 25:1 or 30:1. In the present study, high C: N ratio (25.76±0.49) was noticed at the initial stage, which decreased gradually with the passage of substrate decomposition (Table 1). The results corroborated with the study of **[14]** within 31days of decomposition, the C:N ratio decreased to 12.17±0.18 the final C:N ratio indicates the high rate of decomposition. The decrease of C:N ratio was the result of transformation of organic carbon in to carbon dioxide, followed by a reduction in the organic acid content. In order to identify the good growing medium for actinomycetes isolates, they were grown on different cultural medium. All the actinomycetes isolates were grow on different medium with some variation by colour. As shown in Table 3, the isolate AAUCS2WG6, AAUCS2PY7, AAUCS2WB8, AAUCS2Y10, AAUCS2GP11 and AAUCS2BB12 exhibited excellent growth on Starch Casein Agar medium, the colour of aerial mycelium showed Whitish grey, Pale yellow, Whitish black, Yellow, Grey pink, Blue black and the colour of substrate mycelium is Dark grey, Light orange, Dark brown, Orange, Grey and Light yellowish respectively. Very Good growth was detected on Starch Nitrate Agar medium. Aerial mycelium is Whitish grey, Pale yellow, Black, Yellow, Grey pink, Blue black, and substrate mycelium Dark grey, Light yellowish, Light black, Yellow, Grey, Dark black respectively. Good growth was detected on chitin agar. Aerial mycelium is Whitish grey, Pale yellow, Whitish black, Yellow, Grey pink, Blue black and substrate mycelium is Light grey, Light yellow, Dark black, Yellow, Light grey and Dark black respectively. Further, the colour of aerial and substrate mycelia produced by the twelve isolates varied with different media (Table 3). Thus, it was concluded on

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the basis of the present and previous studies that the nutrient compositions of the medium greatly influence the growth and morphology of organisms **[10]**. According to **[23]** the colour of aerial mycelium, the most of antifungal isolates were found to be gray series and classified them in to five groups: blue, violet, yellow, red and brown. Actinomycetes isolates grew well at 28°C on Starch Casein agar (optimal medium) producing pinpoint to medium sized; slow growing, powdery, and irregular to regular, flat to raised colonies possessing an earthy odour characteristic of actinomycetes. On Starch casein agar isolates produced white coloured aerial spore mass and yellow coloured substrate mycelium **[20]**. All the isolates namely AAUCS2WB8, AAUCS2WG6, AAUCS2PY7, AAUCS2GP11, AAUCS2BB12 and AAUCS2Y10 showed positive results in starch hydrolysis (Table 4). In the nitrate reduction test, AAUCS2WG6, AAUCS2PY7, AAUCS2WB8 and AAUCS2GP11 gave positive results except AAUCS2Y10 and AAUCS2BB12 which is negative. The four isolates showed positive result in gelatin liquefaction (Table 4). A study was done by **[17]** all the isolates were found to be gram positive organism and they showed a branched mycelium in their cell morphology similar to fungal characters. Most of the isolates were efficient in hydrolyzing starch except a few strains, but catalase was positive in all the isolates. Gelatin hydrolysis and starch hydrolysis showed a positive result in majority of the isolates. Based on the result of morphological, cultural and biochemical characteristics it is observed that the isolated actinomycetes strains, AAUCS2WB8, AAUCS2WG6, AAUCS2PY7 and AAUCS2GP11 belonged to the genus Streptomyces (4 isolates) and the two isolates namely AAUCS2Y10 and AAUCS2BB12 were identified as genus Sacharomonospora (2 isolates) as compared to those of the actinomycetes described in Bergey's Manual of systematic Bacteriology **[34]**. The finding of the present study showed that mushroom compost is rich with actinomycetes so, the diversity of both mesophilic and thermophilic actinomycetes in mushroom compost could be further studied.

CONCLUSION

The rate of discovery of new bioactive compounds from existing genera obtained from common soil has decreased So, it is critical that novel actinomycetes from unexplored habitats such as marine, hot spring be pursued as sources of novel antibiotics and others bioactive compounds. Therefore, screening, isolation, and characterization of promising strains of actinomycetes producing potential antibiotics and other therapeutics have been a major part of research. Searching for unique actinomycetes that produce an essential component in natural product-based drug is becoming more and more interesting and meaningful. Hence, the findings of the present study showed that mushroom compost is rich with actinomycetes that have great potential to produce metabolite against antimicrobial activity. Therefore, the diversity of the mesophilic and thermophilic actinomycetes in mushroom compost and methods for large scale production of potent antibiotic producing actinomycetes isolates could be further studied.

REFERENCES

1. *Alharbi, S.A., Arunachalam, C., Murugan, A.M. and Wainwright, M. (2012). Antibacterial Activity of Actinomycetes isolated from terrestrial soil of Saudi Arabia. Journal of Food, Agriculture & Environment.10 (2):1093- 1097.*

2. *Atta, H.M., Haroun, B.M.and Khalifa, M.A. (2011).Physico-chemical characteristics of vernamycin -A antibiotic biosynthesis by Streptomyces SP-AZ-SH-29. Journal of Saudi Chemical Society.15:247-255.*

3. *Attimarad, S.L.,Ediga, G.N.,Karigar, A., Karadi,R., Chandrashekhar, N. and Shivanna,C.(2012). Screening, isolation and purification of antibacterial agents from marine actinomycetes. International Current Pharmaceutical Journal.1 (12):394-402.*

4. *Basavaraj,N.K., Chandrashekhara, S., Shamarez, A.M., Goudanavar,P.S .and Manvi, F.V. (2010). Isolation and Morphological Characterization of Antibiotic Producing Actinomycetes. Tropical Journal of Pharmaceutical Research.9 (3):231-236.*

5. *Bergey's Manual of Systematic Bacteriology, volume four.2000. Actinomycetales, 9th edition.*

6. *Bernal,M.P., Paredes, C.,Sanchez, M.A.andCegarra, J. (1998). Maturity and stability parameters of composts prepared with a wide range of organic wastes. Bioresour Technol.63:91-99.*

7. *Edwards, C. (1993). Isolation Properties and*

Mulatu et.al / UJPSR / 2 (2), 2016, 1-11
e ISSN: 2454-3764
Print ISSN: 2454-3756
DOI: 10.21276/UJPSR.2016.02.02.20

Potential Applications of Thermophilic Actinomycetes. Applied Biochemistry and Biotechnology.42:161-179.

8. *Finstein, M. S. and Miller, M.C. (1985). Composting of Agricultural and other Wastes. Elsevier Applied Science, London, pp.13.*

9. *Gardner, W. H. (1986). Water Content. In: Methods of Soil Analysis, Part 1 Physical and Mineralogical Properties, pp. 493-541 (Klute,A.,edn.).American Society of Agronomy Inc. and Soil Science Society of America, Inc.,Madison.*

10. *Gesheva, V. (1993).Structure of the Streptomyces hygroscopicus 111-81 population and characteristics of its variants. Actinomycetes. 4: 65-72.*

11. *Goodfellow,M. and Williams, S.T.(1983).Ecology of Actinomycetes. Ann. Rev.Microbial. 37:189-216.*

12. *Goodfellow, M. and O'Donnell, A.G. (1989). Search and discovery of industrially-Significant actinomycetes. Proceeding of the 44th Symposium on Society for General Microbiology, (SCGM'89), Cambridge University Press, Cambridge, pp: 343-383.*

13. *Gray, K.R.,Sherman, K.andBiddlestone, A.J. (1971).A review of composting Part 2- The practical process. Process Biochemistry.6 (10):22-28.*

14. *Hadas,A. and Portnoy, R. (1994). Nitrogen and carbon mineralization rates of composted manures incubated in soil.J.Environ.Qual.23:1184-1189.*

15. *Harisba,S. (2006). Biotechnology Procedures and Experiments Hand book. Infinity Science Press LLC; Hingham, New Delhi.pp:233-249.*

16. *Hassen, A., Belguith, K., Jedidi, N., Cherif, M. and Boudabous, A. (2002). Microbial Characterization during composting of municipal solid waste. Proceedings of International Symposium on Environmental Pollution Control and Waste Management. pp: 357-368.*

17. *Hemashenpagam, N. (2011). Purification of secondary metabolites from soil actinomycetes. International Journal of Microbiology Research.3:148-156.*

18. *Jackson,M.L.(1958). Soil Chemical Analysis. Prentice Hall, Inc., Engle wood Cliffs. New Jersey, pp: 183-204.*

19. *Kaviyarasi,K.,Kanimozhi,K.,Madhanraj,P., Panneerselvam,A.andAmbikapathy,V. (2011). Isolation, Identification and Molecular Characterization of Phosphate Solubilizing Actinomycetes Isolated From The Coastal Region of Manora, Thanjavur. Asian J. Pharm.Tech.1:119-122.*

20. *Lakshmipathy, D., T. and Kannabiran, K. (2009). A Morphological, Biochemical and Biological Studies of Halophilic Streptomyces sp. Isolated from Saltpan Environment. American Journal of Infectious Diseases.5 (3):200-206.*

21. *MAFF, (1986). Ministry of Agriculture, Fisheries and Food. The Use of Sewage Sludge on Agricultural Land. MAFF/ADAS booklet 2409. Revised 1987.MAFF Publications, London.*

22. *Mary, B. and Recous, S. (1994). Measurement of nitrogen mineralization and immobilization flues in soil as a means of predicting net mineralization. European Journal of Agronomy.3:1-10.*

23. *Mohamed, T.S. (1995). Production of antibiotics and pigments by some pigmented soil Streptomycetes. M.Sc. Thesis,Ain shams university, Egypt.*

24. *Mulatu and Dawit. (2016). Screening of bioactive compounds from actinomycetes isolated from compost prepared for mushroom growth against candida albicans. International Journal of Innovative Pharmaceutical Sciences and Research, 4 (8): 887-899.*

25. *Okami,Y. and Hotta, K. (1988). Search and discovery of new antibiotics. In: Actinomycetes in Biotechnology.pp:37-67. (GoodFellow, M., Williams, S.T. and Mordarski, M.,eds.). Academic press, London.*

26. *Peters, S., Koschinsky, S.,Schwieger ,F.and Tebbe, C. (2000). Succession of Microbial Communities during Hot Composting as Detected by PCR Single-Strand-Conformation Polymorphism-Based Genetic Profiles of Small-Subunit Rrna Genes. Appl. Environ. Microbiol.66 (3):930-936.*

27. *Pincelot,S.,P.(1975). Biochemistry and Methodology of Composting. Connecticut Agr. Expt Sta. Bulletin 754.*

28. *Sahilemedihin Sertsu and Taye Bekele. (2000). Procedures for Soil and Plant Analysis. Technical Paper. National Fertilizer Sector Project .Addis Ababa, Ethiopia.*

29. *Salar, R.K. and Aneja, K.R. (2007). Significance of thermophilic fungi in mushroom Compost preparation: effect on growth and yield of Agaricusbisporus. Journal of Agricultural Technology.3 (2): 241-253.*

30. *Thomas, G.W. (1996). Soil pH and Soil Acidity. In: Methods of Soil Analysis,pp: 475-491. (Sparks, D. L.edn.).American Society of Agronomy Inc. and Soil Science Society of America, Inc.,Madison.*

Mulatu et.al / UJPSR / 2 (2), 2016, 1-11
e ISSN: 2454-3764
Print ISSN: 2454-3756
DOI: 10.21276/UJPSR.2016.02.02.20

31. *Vengadesh, P.K., Sundaramoorthi, C., Gupta, S., Karthick, K. and Tamilselvi, N. (2011).Production and characterization of antibiotics from soil isolated Actinomycetes. IRJP.2 (4):114-118.*

32. *Waites, M.J., Morgan, N.L., Rockey, J.S. and Higton ,G. (2001). Industrial Microbiology: An Introduction. Blackwell Science Ltd; School of Applied Science, South Bank University, London, UK.pp:226-240.*

33. *Williams, S.T. and Davies, F.L.(1965).Use of antibiotics for selective isolation and enumeration of Actinomycetes in soil. Journal of General Microbiology.38:251-261.*

34. *Williams, S.T., Sharpe, M. E. and Holt, J.G. (1989). Bergey's Manual of Systematic Bacteriology. Vol. 4, William and Wilkins Co., Baltimore.*

35. *Woods End Research Laboratory, (1998). Interpretation of Waste and Compost Tests, Journal of the Woods End Research Laboratory, 1 (4):1-6.*

36. *Yalinkilic, M.K.,Altun, L., Baysal, E. and Demirci, Z.(1994). Development of mushroom cultivation techniques in Eastern Black Sea Region of Turkey. Project of The Scientific and Technical Research Council of Turkey. pp: 377.*

37. *Yi ne,Z. and Amsaveni, S. (2012).Isolation, Screening and Characterization of Antibiotic Producing Actinomycetes from Rhizosphere Region of Different Plants from a Farm of Sungai RamalLuar, Malaysia. Journal of Advanced Biomedical and Pathobiology. 2(3):96-107.*