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Cultivation technology and antibacterial activity of Agaricus bisporus (U-03)

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ABSTRACT

Agaricus bisporus, being one of the most commonly white button mushrooms contributes about 40-45% to the world mushroom production. Presence of viable cells were measured in terms of viable count or CFU, which exhibit progressive declination in count of thermophilic fungi from initial stage of composting. Similarly, declination of pH ranges from 8.3-7.1 alkaline pH in initial stage was due to microbial decomposition of organic acids and subsequent release of ammonia through mineralization of organic nitrogen sources, declination of weight from 1.465 Kg. to 0.491 Kg and temperature from 44-34°C was due to extensive utilization of compost as nutrient by inhabiting thermophilic fungi. Result obtained exhibit highest cellulose content in raw material of which horse manure exhibit higher cellulose content then wheat straw, horse manure 2.052mg and wheat straw 1.044mg. Highest cellulolytic activity was observed in horse manure followed by wheat straw in basal material. Horse manure 2.167micromoleglucose released/ml/hr and wheat straw 1.014micromoleglucose released/ml/hr. Highest antimicrobial activity was observed in methanol extract (14mm) against B. cereusas compared to ethanol extract (7mm). Same results obtained for S.aureus(20mm) and B. cereus (14mm) in methanol extract. Least microbial growth inhibition was observed in S. aureus (25mm) followed by B. cereus (7mm) with respect to control in ethanol extract. No antimicrobial activity observed in P. aeruginosa in ethanol but least inhibition was observed in methanol extract.

1. Introduction

Agaricus bisporus, being one of the most commonly white button mushrooms contributes about 40-45% to the world mushroom production (Flegg, 1992). A. bisporus is a low temperature species requiring 23 ± 20 C for its vegetative growth and 16 ± 20^{0}C for its fruiting (Singh, 2011) and widely consumed mushrooms in the world is cultivated in more than seventy countries. Morphologically, A. bisporus bears 3-16 cm cap, convex to broadly convex, dry, smooth or with pressed-down fibers or small scales, white in some varieties, brown in others, with gills which are free from the stem, close, pinkish to pinkish brown at first, becoming dark brown to blackish. The stem measures around 2-8 cm long, 1-3 cm. thick, study, more or less equal, smooth or with small scales below the ring, white, often bruising brownish, with a ring that sometimes disappears in maturity. A. bisporus flesh is white and firm, usually bruising and staining brownish, and give pleasant odor and taste. Microscopically, the brown spore print exhibits as spores 5.5-8.5 x 4-6.5 μ, elliptical, smooth with 2-spored basidia (kuo, 2004). A.bisporus mushrooms are well-known for their nutritional and medicinal values. They are also valued for waste management as most of them grow on lignocellulosic materials of agricultural origin, forest litter and garden litter. A. bisporus is a source of protein (about 40% on dry basis), ergo sterol, a precursor of vitamin D, several minerals, carbohydrate and fat. The major minerals detected in it are sodium, potassium and phosphorus. Agaricus bisporus has substantial amounts of Vitamin D which is good for our health. Eating the mushroom raw is discouraged because of the presence of carcinogenic hydrazine derivatives, albeit in small quantities. A. bisporus

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mushroom contains Potassium and Sodium. The diet dependent statistically significant decrease of incidence of the disease has been reported in women diagnosed with breast cancer. It exhibit anti-microbial properties and anti-oxidant properties as evidenced by inhibitory properties of its extract against some gram positive as well as gram negative bacteria**.** Different methodologies such as microdilution method, the disk diffusion method, the agar streak dilution method based on radial diffusion method and a method with the incorporation of the extract in culture medium and further determination of colonies have been used to asses antimicrobial activity of A. bisporus extracts and compounds. [1–3]

The above cited description points to the nutritional and medicinal values and subsequent studies on Agaricus bisporus with reference to its cultivation and antimicrobial properties however, there is hardly such report available in the vicinity of industrial hub of Himachal Pradesh. Thus, present piece of work highlights the mushroom cultivation under prevalent environmental conditions using regional resources and local bacterial isolates.

2. Material and Methods

2.1. Cultivation procedure

2.1.1. Tissue culture (Singh, 2006)

Under aseptic conditions using laminar flow, young basidiocarp was cleaned with sterilized distilled water and dipped into 0.1% mercuric chloride or 2.5% sodium hypochlorite solution for 1 min or washed with 85% ethanol and air dried then button mushroom split open longitudinally from centre and vegetative mycelial bits are cut from the collar region (junction of pileus and stipe) after that bits are inoculated centrally in starch/Malt extract agar (MEA) plates and inoculated plates are incubated at 25 ± 2^0C in a BOD incubator. Within 4-5 days the new mycelium growing over the media was observed. The pure cultures were made by carefully transferring the young mycelium from growing edge of the colony from petriplates to test tubes and again incubated at 25 ± 2^0C for 10-14 days. Three replica of each media were maintained. [4–6]

2.2. Preservation of culture (Singh, 2006)

The full growth culture tubes were stored at cooler temperatures, i.e., at 4^0C to 7^0C in a refrigerator or cold room till further use.

2.3. Spawn preparation (Vijay, 1996)

About 300 g prepared substrate (boiled wheat grains mixed with gypsum and chalk @ 2.0% & 0.5%) was filled in Erelenmeyer flasks upto 2/3 volume and plugged with non-absorbent cotton. The plugs are covered with aluminum foil. These filled bottles were autoclaved at 15 psi pressure. After autoclaving bottles were left in the room for 24-48 hrs for cooling. A piece of mycelium (pure culture) grown in Petri plates was aseptically transferred to these bottles and inoculated bottles were incubated at 25 ± 1^0C. These bottles are gently shacked on 5^{th} and 10^{th} day for distributing the inoculum evenly in the bottles. After 10^{th} day grains were fully colonized with button mushroom mycelium (U-03). The fully colonized grains were then used as spawn or inoculants in compost.

2.3.1. Mushroom composting

2.3.1.1. Compost ingredients / Formulations (IARI). White button mushroom (A. bisporus) requires a well composted substrate for its growth. Compost was product of aerobic fermentation of carbon and nitrogen source supplemented in the form of agricultural waste and manure. Hence, a specific composition or formulation was required for cultivation of A. bisporus (U-03). A standard formulation given by IARIwill be use for compost production.

Compost ingredients	**Quantities (Kg.).**
Horse manure	1.0
Wheat straw	0.35
Urea	0.005
Gypsum	0.03

2.4. Methodology for substrate preparation (Pathak, 2014)

Wheat straw was dip in tub containing water having trace amount of formalin and bavistin in it, for 24-48 hrs to achieve 70-73% moisture. After soaking all ingredients were mixed in wheat straw and then mixed composite materials were filled in sterile beakers @ 1 Kg. Whole sets were incubated at 47 ± 1^0C and after filling periodic turning of 13 and 12 were given to all the sets. So that, malodorous gases get eliminate from the compost and compost become aerobic which made possible to grow aerobic microorganism's mainly thermophilic microorganisms in compost. This made compost selective for the growth of A, bisporus.

2.5. Spawning and harvesting (Vijay, 1996)

2.5.1. Spawning

After completion of composting, compost of both sets were spawned with desired inoculants (A. bisporus, U-03). The spawned compost of both sets was incubated at 25 ± 1^0C for 12-15 days. After completion of spawn run, a layer of sterile casing made up of 1:3 ratio of farmyard manure and coir pith were placed on the top surface of the compost which facilitate the fruiting bodies formation. After casing again both sets of compost were incubated at 25 ± 1^0C till

the mycelium of compost emerges to the casing layer. After that small pin head structure of nascent mushroom fruiting bodies get appeared on the casing layer. Fruiting bodies formation takes place after 7-8 days of casing.

2.5.2. Harvesting

When fruiting bodies appeared on the compost bag as pin heads the temperature of the compost bag were made lower down to 18 ± 1^0C, humidity 80-90% and ample of fresh air were introduced to the sets. At this temperature mushroom develop which later on get harvested by gently twisting. After harvesting yield were recorded on digital balance.

2.6. Physiochemical investigation

2.7. Physical parameter (Vijay, 1996)

2.7.1. Moisture

Representative composite compost sample of two sets were weighed in a clean dry pre-weighed glass Petri plates. The samples were then dried in hot air oven at 45 $\pm2^0$C till complete dryness and constant weight of each plate get achieved. The difference in weight was expressed as moisture percent and it was calculated by using formulae as given below.

$$Moisture\% = \frac{Wet\ weight - Dry\ weight}{Wet\ weight} X\ 100$$

2.7.2. pH

Ten grams of composite sample of each set was suspended in 100 ml. of distilled water and shaken for some times. The pH of suspension was determined by using digital pH meter.

2.7.3. Temperature

The temperature of both sets was daily recorded with the help of mercury thermometer.

2.7.4. Weight loss

Weight loss as weight of beakers was recorded from filling to end of composting and is estimated on digital pan balance and calculated by using formula as given below.

$$Weight\ loss\ \% = \frac{Weight\ at\ zero\ days \tilde{} Weight\ during\ turning}{Weight\ at\ zero\ day} X\ 100$$

2.8. Biochemical parameter

2.9. Estimation of cellulose by anthrone method (Updegroff, 1969)

2.9.1. Anthrone reagent

Dissolved 200 mg anthrone in 100 ml. of ice cold concentrated sulphuric acid. Reagents were freshly prepared.

2.9.2. Acetic/Nitric acid reagent

Mixed 150 ml. of 80% acetic acid and 15 ml. of concentrated sulphuric acid.

2.9.3. 67% sulphuric acid

2.9.3.1. Procedure. 3 ml. acetic acid/nitric acid reagentswere added in 1.0g. Of dried and grounded wheat straw, horse manure and compost samples in a test tube. Test tubes were then kept in boiling water bath for 30 min. After that test tubes were cooled at room temperature and then centrifuged at 10,000 rpm for 15-20 min. After centrifugation supernatant were discarded and residues were washed with distilled water. Then after 10 ml. of 67% concentrated sulphuric acid were added in each test tube and allowed the test tubes to stand at room temperature for 1h. Now one ml. of above solution were taken in other test tube and diluted by adding 99 ml. of distilled water in it. Again one ml. of diluted sample were taken in other test tubes and 9 ml. of anthrone reagent were added in it. After adding anthrone reagent test tubes were boiled in water bath for 10 min. cooled and then measure the colour at 630 nm. Blank containing 1 ml. distilled water and 9 ml. anthrone reagents were used as control. Estimation of cellulose was done by making standard curve corresponding 40-200μg. of cellulose and then calculate the cellulose % by using formulae given below.

$$Total\ \ cellulose = \frac{Cellulose\ value\ from\ graph(\mu g.)}{Aliquot\ of\ the\ sample\ used} \times \frac{Total\ vol.\ of\ extract\ prepared}{Weight\ of\ the\ sample\ taken} \times \frac{1}{100}$$

2.9.4. Estimation of cellulase (FPase by using standard protocol (Miller, 1959)

2.9.4.1. Preparation of enzyme extract. One gram of basal material and compost were suspended in phosphate buffer of pH 7.0 & 8.0 and centrifuged at 1000rpm for 10 min. Supernatant were taken for enzyme studies.

2.9.5. Methodology for assay of total cellulose (FPase)

2.9.5.1. 1M Phosphate buffer (pH 7.0 & 8.0) . The Buffer solution was prepared as follows.

1. 0.2M solution of monobasic sodium phosphate (27.8g. in 1000 ml.).
2. 0.2 M solution of dibasic sodium phosphate (53.65g. of $Na_2HPO_4.7H_2O$ or 71.7g. of $Na_2HPO_4.12H_2O$ in 1000 ml.).

x ml. of A + y ml of B diluted to 200 ml

x ml.	y ml.	pH
39.0	61.0	7.0
16.0	54.0	8.0

Reagents used in enzyme estimation
Di-nitro salicylic acid reagent (DNS)

2.9.6. Rochelle's salt

40% sodium potassium tartrate were made.

Chemicals	Quantities
2,3 DNS	1%
Phenol	0.2%
Na_2CO_3	0.05%
NaOH	1%

2.9.7. Standard glucose solution

One gram of A.R. grade glucose (Dextrose) was dissolved in 100 ml. of distilled water. This stock solution contains 10mg. glucose/ml. From the stock solution standards 1,2, 3 and 4 solutions containing 0.0125 mg., 0.025mg., 0.05 mg. and 0.1 mg./ml. solutions were prepared.

2.9.8. Substrate for enzymes

40 mg. whatman filter paper No. 1 was suspended in 100 ml. of 0.1 M phosphate buffer of pH 7.0 and 8.0.

2.9.9. Estimation of glucose

Glucose was estimated by using DNS method. Four standard glucose solutions were used for preparing standard curve. Standard glucose solution (3 ml.) and 3 ml. DNS were added in test tube and then test tube was kept in boiling water bath for 30 minutes and then cooled at room temperature. 1 ml. of Rochelles salt was added in test tubes during cooling. Total volume was made up to 10 ml. by adding distilled water. The optical density of solution was measured at 540 nm. Three replications were maintained for each standard solution. A control with distilled water was also run simultaneously. The standard curve was used to estimate the amount of glucose released in the assay of cellulose.[7–9]

2.10. Enzyme assay

2.11. Total cellulase/exoglucanase

Reaction mixture containing 2 ml. of substarte and 1 ml. of enzyme solution were added and it was incubated at 47 ± 1^0C for 30 minutes a control without enzyme sources were also run simultaneously. After incubation 3 ml. of DNS was added in the solution and the reaction mixture was kept in boiling water bath for 30 minutes. Prior to cooling 1 ml. of 40% Rochelle salts were added in solution. Volume of reaction mixture was made up to 10 ml. by adding distilled water in it. A control set without enzyme source was run parallel to this. The enzyme activity was calculated in the units of μmoles glucose released/ml./hr.

Calculation of enzyme activity

$$Enzyme\ activity = \frac{\triangle OD\ X\ Standard\ factor\ X\ Dilution\ factor}{Incubation\ time\ X\ Enzyme\ volume\ (ml).}$$

Screening of anti bacterial activity property of A. bisporus**.**

Isolation and identification of gram positive and gram negative bacteria from sewage sample

2.12. Collection of samples: (Willey, Sherwood and Woolverton 2011

For the isolation of different gram positive and gram negative bacteria sewage samples were collected from different sewage treatment plants in sterile beaker and collected samples were transferred to sterile plastic bags in aseptic conditions.

2.13. Isolation of different gram positive and gram negative bacteria from sewage samples: (Willey, Sherwood and Woolverton. 2011).

For isolation of bacteria from sewage samples two media were taken for isolation of bacteria are. Media composition is given in appendix.

1. NAM (Nutrient agar medium) / NBM (Nutrient broth medium).
2. MA (Mac conkey Agar medium) / MB (Mac conkey broth medium).

2.13.1. Procedure

Serial dilution agar plating method is one of the most commonly used procedure for the isolation of microorganisms from sewage samples. In this 1ml of sewage sample was added to 9ml of distilled water to make the final volume 10ml. Serial dilutions were done ranging from10^{-2}, 10^{-3},.....10^{-7} by pipetting 1ml into additional dilution blanks. Finally, 1ml aliquot of various dilutions was added to sterile petriplates having 15ml of sterile, cool, molten nutrient agar media. The dilutions 10^{-4} to 10^{-7} were selected for enumeration of bacteria. Upon solidification, the plates were incubated in inverted position for 3-4 days at 37 ± 1°C after incubation, different types of colonies were appeared on plates. Three replication of each dilution were maintained.

2.14. Identification of microorganisms: (Holt et al, 1994)

After inoculation the isolated colonies appeared on medium were identified on the basis of Bergy's mannual of determinative bacteriology. The isolated microorganisms were identified on the basis of colony morphology, texture, Gram staining, Endospore staining and biochemical tests.

2.15. Colony morphology

Different types of isolated microorganisms exhibited different types of colonies on surface of agar which were later identified externally by seeing characters like shape (circular, dot like, irregular etc.), colony elevation (thin film, raised, convex etc.), colony margins (entire, crenate, dentate, lobate etc.), optical density (transparent, tanslucent, opaque etc.).

2.16. Texture

Slimy and glistering colonies were seen on the medium.

2.17. Staining methods

2.17.1. Gram staining

Further identification of isolated bacterial colonies as Gram positive and Gram negative were done by Gram staining. A thin smear of isolated bacterial culture was made on a clean slide and heat fix was done. The smear was covered with crystal violet for 30 seconds and then washed the slide with distilled water. Gram's iodine was added to the smear for 60 seconds and washed with 95% ethyl alcohol. Then again washed the slide with distilled water. Counter staining was performed by applying safranin to the smear for 30 seconds. After 30 seconds, slide was washed with distilled water, blot dried with absorbent paper and slide was air dried. Then slide was examined under microscope by using immersion oil.[9,10]

2.17.2. Endospore staining

Further confirmation for presence or absence of endospore, endospore staining was done by making smear of isolated bacterial culture on a clean slide and heat fixing was done. The slide was then flooded with malachite green and steamed for 5minutes, more stain added time to time. Then slide was washed with water. Safranin was used as counter stain for 30 seconds and slide was again wash with water. Slide was blot dried and examined under microscope.

2.18. Biochemical tests

2.18.1. Catalase test

Trypticase soy agar slants were prepared and inoculated with isolated bacterial culture. Slants with culture were inoculated 35°*C* for 24–48 hrs. After incubation, 3-4 drops of hydrogen peroxide were added to the growth of each slant culture. Composition of medium is given in Appendix.

2.18.2. Oxidase test

Oxidation and fermentation glucose media(OF glucose media). was autoclaved in tubes and then cooled for some time. One tube was inoculated with isolated bacterial culture and other tube was not inoculated which was used as a control. Then liquid paraffin was poured over the medium to form a layer about 1cm in depth in the tubes. Then tubes were incubated at 35 ± 1^0C for 24-48 hrs. Composition of medium is given in Appendix.

2.18.3. Mannitol fermentation test

Mannitol salt agar media plates were prepared and streaked with isolated bacterial culture. These plates were incubated at 37±1°C for 24-48 hrs. Composition of given medium is given in Appendix.

2.19. Purification and preservation of isolated microorganisms: (Willey, Sherwood and Woolverton 2011

Isolated and identified colonies were maintained on slants and kept at 4 ± 1^0C till further use. Slants were revived periodically to maintain the colonies in active conditions.

2.20. Screening of anti-bacterial activity of A bisporus

2.20.1. Collection of A. bisporus(U-03)(Vijay, 2006).

Fresh and healthy fruiting bodies of A. bisporus (U-03) were collected from the beakers and after that fruitbodies were kept at 47 ± 1^0C in hot air oven to get fully dried till constant dry weight.

2.20.2. Preparation for extract(Sagar et al., 2012).

Dried fruiting bodies of A. bisporus(U-03) were weighed to know the moisture content and then grinded in fine powder in mortar pestle. Powder of one gram dried fruit bodies were suspended in 9 ml. ethanol, methanol and distilled water. Extraction was accomplished by stirring solution at 150 rpm for 24h at 20^0 C. The broth was then centrifuged at 10,000rpm for 10 minutes and the supernatant was filtered by using whatman No. 1 filter paper and extracts were collected in pre sterilized micro-centrifuge vials. Extracts were then stored at 4^0C for study of antibacterial activity.

2.20.3. Screening of anti-bacterial activity of A. bisporus(U-03).(Bauer,. et al., 1966)

In vitro anti-microbial susceptibility tests are performed by using identified pathogenic microorganisms following agar well diffusion method. NAM was used throughout the investigation for the growth of microorganisms. Medium was autocalved at 121^0C for 30 minutes. The plates were left overnight at 37 ± 1^0C for pre incubation to check for any contamination to appear. Then after freshly grown bacteria were seeded on the medium to prepare bacterial lawns. Inoculated plates were incubated at 37^0C for 24 hrs. to develop lawn on the plate and then after two agar wells of 8mm diameter were made opposite to each other. One well serves as control and other serve as test. 10 μl. of extract were pipetted in one well and its and its respective solvent without extract were pipetted in other well. After pipetting plates were kept at 20 ± 1^0C for 1 hrs. so that extract get absorb in the medium. After that plates were incubated at 37 ± 1^0C for 24 hrs. to see the effect of extract on bacterial lawn and result thus obtained get compared to control treatment.

Table 1: Physiochemical andbiological parameter of Compost

S.No.	Ingredients	Turnings	pH	C.F.U/ml	Weight loss (Kg.)	Temperature(^{0} C)
1	Compost	1^{st}	8.3	0.24	1.465	39
2	Compost	2^{nd}	8.3	0.22	1.410	40
3	Compost	3^{rd}	8.2	0.21	1.378	42
4	Compost	4^{th}	8.1	0.20	1.310	44
5	Compost	5^{th}	7.9	0.17	1.302	43
6	Compost	6^{th}	7.8	0.13	1.180	42
7	Compost	7^{th}	7.7	0.13	1.076	42
8	Compost	8^{th}	7.7	0.12	0.988	41
9	Compost	9^{th}	7.5	0.10	0.860	41
10	Compost	10^{th}	7.4	0.07	0.760	38
11	Compost	11^{th}	7.3	0.03	0.627	34
12	Compost	^{12th}	7.1	0.03	0.491	34

3. Results and Discussion

3.1. Compost preparation and its physiochemical and biological parameter

Compost is the substrate used for cultivation of A. bisporus. A. bisporus utilize compost as nutrient source carbohydrate and protein part of compost serve as carbon and nitrogen source for A. bisporus. A. bisporus compost was prepared by using ingredients and methods as mention in para 1d.of section A of materials and methods. Moisture of compost was adjusted to 70-73% and composting was done under in vitro condition or lab condition. About one kg. of compost was filled in pre weighed beakers and set of filled beakers were incubated at 47+1^{0}C for 12-13 days. Periodical turnings (12-13 turnings) of compost were given from zero day(filling day) to maturation stage (at the time of spawning) and compost get matured or get ready for spawning after 21 days. Turning is given after two days interval from filling so that ammonia and other malodorous gases generated due to fermentation must get escape and make compost aerobic so that proliferation and colonization of thermophilic microorganism get well established in compost. Dominance of these fungi in compost at early stage of composting make compost nutritive and selective for the growth of A. bisporus. Several workers also reported the dominance of thermophilic microorganisms in compost in early stages which favors the growth of A. bisporus in compost. (Waksman et al.,1939, Vijay, 1996, Pathak, 2014). These thermophilic microorganisms utilize cellulose and proteins as carbon and nitrogen. Thermophilic microorganisms get carbon and nitrogen sources from agricultural residue and manure supplemented at the time of composting as raw material. Lille amount of Urea and gypsum is also added in compost. Addition of this component in compost maintains the C/N ratio and pH of compost. Due to the presence of high amount of carbohydrate and protein compost become favorable of inhabiting microorganism. In this case thermophilic microorganisms well colonized in early stage of composting and later starts decline. Declination in population is due to the saturation or less availability of limited nutrition in compost. Result as dominance or presence of viable cells were measured in terms of viable count or CFU. Count of thermophilic fungi gradually decline from initial stage to final stage of composting. Similarly, declination in pH, weight and temperature of compost were also observed from initial to final stage of composting. Declination of pH ranges from 8.3-7.1 alkaline pH is due to the microbial decomposition of organic acids and subsequent release of ammonia through mineralization of organic nitrogen sources. Rapid declination of Ph from initial to final stage of composting is due to the utilization of animal manure and nitrogen fertilizer in the compost and moreover scarification of compost which result in neutral pH and sweet smell of the compost. Compost at this pH favors the growth of A. bisporus hence spawning or adding inoculants at this pH is recommended. Hence at this stage U-03 was spawned in compost. Declination of weight from 1.465 Kg. to 0.491 Kg. and temperature from 44 -34^{0}C is due to extensive utilization of compost as nutrient by inhabiting thermophilic fungi. Result observed in temperature study was quite surprising in initial stage of composting temperature of compost was low later or at room temperature later on it started rising and maximum in fourth turning (44^{0}C) then after it started stabilizing and it remains stable till seventh turning then after it started declining and stabilized at maturation or twelfth turning(34^{0}C). The up and down fall in temperature is thought to be of gases accumulated in compost during composting and respiration of thermophilic fungi population in compost which get released during turning. Several workers also reported the same (Vijay, 1996, Sharma, 1991, Satyanarayanan, 1987). Result as CFU count, pH, Weight loss and temperature is depicted in given (Table 1).

3.2. Biochemical investigation of raw material/basal material and compost

3.2.1. Cellulose determination

Wheat straw and horse manure is the main basal material used for compost preparation. This material have cellulose which serve as carbohydrate source for growing thermophilic microorganisms during composting and A. bisporus in compost and these fungi proliferate in compost by utilizing this component. A. bisporus act as mycophagous as it utilize pre dominantly present thermophilic fungi along with degraded cellulosic material. Hence to know the succession of cellulose or total amount of cellulose present in compost from first turning to final stage of composting and during spawn run a cellulose estimation was performed in basal raw material (Wheat straw & horse manure), in different turnings of compost and during spawn run by using standard protocol given in para 3a(i) in chapter-2. Result obtained exhibit highest cellulose content in raw material of which horse exhibit higher cellulose content then wheat straw. Cellulose concentration in compost measured at the time of first turning was high as compared to first, sixth and tenth turnings of compost but low as compared to raw material. Lowest concentration of cellulose in each successive turning as compared to basal material is due to the utilization of cellulose by pre dominant microorganisms present in compost during composting and A. bisporus in mature compost. Degradation of cellulose during composting make readily available sugar in substrate which later used by A. bisporus for their growth. Result as total cellulose concentration of raw material and in compost is calculated on the basis of standard graph. Result given in Tables 2, 3 and 4.

3.2.2. Standard cellulose

A straight line plotted on standard graph at different concentration of cellulose. Intersection appeared on each point of cellulose concentration. Optical density of std.3 was later used for calculation of total cellulose concentration (Table 2).

Total cellulose concentration on basal material, different succession of composting and during spawn run.

3.2.3. Cellulase assay

Cellulose is commonly degraded by an enzyme called cellulase. Cellulase is secreted in compost by inhabiting thermophilic microflora. The end product of cellulose is glucose. Thus release of glucose in basal material and compost was estimated by Filter paperase method (FPase) or exo glucanase by using standard protocol (Miller, 1959). Detail methodology of protocol is given in ——- of chapter 3. Similar trend as total cellulose concentration was observed in cellulase assay. Highest cellulolytic activity was observed in horse manure followed by wheat straw in basal material. Downward fall of cellulolytic activity was exhibited in different turnings of composting and least was exhibited during spawn run. Hence from the observation it was concluded that horse manure is the chief source of glucose which was readily utilized during composting by different thermophilic fungi this results in progressive down fall of glucose in compost. However small elevation of glucose concentration was observed in second turning it was due to the highest degradative activity of densely populated thermophilic fungi. After second turning declination of FPase activity was exhibited. Declination of glucose concentration adversely affect the population of growing thermophilic fungi which results in low count of CFU from Ist turning to spawning, But this will not effect on growing of button mushroom as button mush room take nutrition from thermophilic microflora and degraded straw based material. Result as micromole glucose released/ml/ hr in basal material as well as in compost at different turnings are given (Tables 5, 6, 7 and 8).

3.2.4. Anti microbial activity of A. bisporus (U-03)

Screening of anti microbial activity of U-03 was observed on two bacterial isolates which were isolated from sewage samples and identified on the basis of morphology, Gram staining and biochemical properties. Detail methodology of antimicrobial assay is gtiven in para——chapter 2. Result as identification of bacterial isolates and inhibition zone formation by U-03 is given (Tables 7 and 8).

From the Table 8 it is clear that highest inhibition was observed in methanol extract as compared to ethanol extract. Highest inhibition was observed in plate inoculated with S. aureus, p.aeruginosa and B. cereus in methanol extract. Least inhibition was observed in S. aureus followed by B.cereus. with respect to control in ethanol extract.

4. Conclusion

From the present investigation it was concluded that agaricus bisporus (U-03) was cultivated on aerobic fermented substrate known as compost. Fermentation in compost was brought by inhibiting thermophilic microflora in it. During fermentation various physiochemical and biological changes were observed it was due to the utilization of substarte as nutrient sources by thermophilic fungi. Colonization of this fungus made compost favourable for the growth of U-03 in compost. U-03 extensively proliferates in compost by utilizing thermophilic microflora along with compost as nutrition sources. Time taken for compost production was 21 days and colonization of mushroom mycelium on compost 7-10 days. Later on investigation was done for screening of antibacterial activity of U-03 against three bacterial isolates viz B. cereus, S. aerus and P. aeruginosa and it was found that the extract of this mushroom was more or less inhibitory to all three bacterial isolates. Hence there is need to exploitation of U-03 along with thermophilic fungi on large scale for

Table 2: Standard curve of cellulose determination

S. No.	Conc.(μl)	D.H_2 o (μl)	Anthrone (ml)	O.D. at 620 nm
1	40	960	10	0.08
2	80	920	10	0.25
3	120	880	10	0.43
4	160	840	10	0.62
5	200	800	10	0.81

Table 3: Total cellulose concentration in basal material

Ingredients	Cellulose concentration(mg).
Horse manure	2.052
Wheat straw	1.044

Table 4: Total cellulose concentration in compost

Compost	Total cellulose concentration(mg).
I turning	0.039
II turning	0.036
III turning	0.027
During spawn run	0.010

Table 5: FPase activity of basal material

Compost basal material	Micromoleglucose released/ ml/hr
Horse manure	2.167
Wheat straw	1.014

Table 6: FPase activity of compost

Basal material	Micromolglucose released/ml/hr
I turning	0.063
II turning	0.079
VI turning	0.022
During spawn run	0.0013

Table 7: Identification of bacteria

S. No.	Organism	Colony morphology (NAM)	Gram stain	Endospore staining	Catalase test	Oxidase test	H_2S Production
1.	Bacillus cereus	White waxy colonies	Rod +ve	+ve	-ve	+ve	-ve
2.	Pseudomonas aeruginosa	NAM changes to bluish green	Rod -ve	-ve	+ve	+ve	-ve
3.	Staphylococcus aureus	Creamish colored colonies	Coccus +ve	-ve	+ve	-ve	-ve

Table 8: Zone of inhibition

Bacillus cereus			
Test	**Control**	**Zone of inhibition (in extract)**	**Zone of inhibition (in control)**
Mushroom extract in methanol	Methanol	14mm	1mm
Mushroom extract in ethanol	Ethanol	7mm	8mm
Pseudomonas aeruginosa			
Methanol+Mushroom extract	Methanol	20mm	29mm
Staphylococcus aureus			
Mushroom extract in methanol	Methanol	20mm	1mm
Mushroom extract in ethanol	Ethanol	25mm	29mm

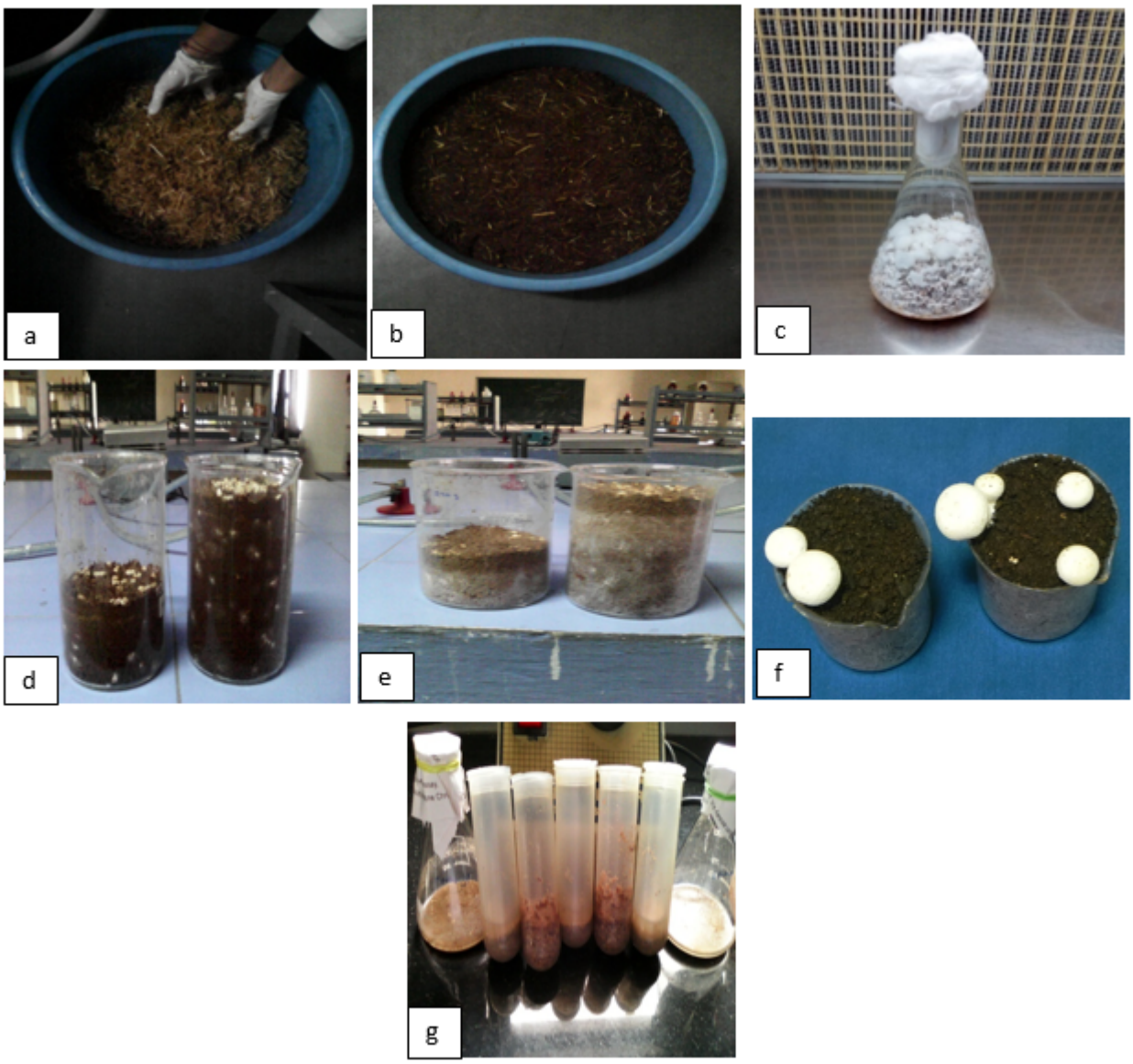

Fig. 1: Compost preparation and fructification of Agaricus bisporus: **a:** Wheat straw; **b:** Compost (horse manure and wheat straw); **c:** Spawn preparation; **d:** Spawning; **e:** Running of mycelium; **f:** Running of mycelium; **g:** Fructification

biodegradation and therapeutic use which is still in infancy stage.

5. Source of Funding

None.

6. Conflict of Interest

None.

References

1. Flegg PB. Future Strategies for Mushroom Production. *Mushroom Res*. 1992;1:13–8.
2. Singh M, Vijay B, Kamal S, Wakchaure GC, editors. Mushroom cultivation, Marketing and consumption. Solan, HImachal Pradesh: Directorate of Mushroom Research (ICAR); 2011.
3. Kuo M. Agaricus bisporus: The common mushroom. MushroomExpert.Com; 2004. Available from: MushroomExpert.Com.
4. Singh SK. Mushroom culture and preservation techniques; 2006.
5. Vijay B. Investigations on compost mycoflora and crop improvement in Agaricus bisporus (Lange) Sing; 1996.
6. Updegeoff DM. Semimicro determination of cellulose in biological materials. *Anal Biochem*. 1969;32(3):420–4.
7. Miller GL. Use of Di nitro salicylic acid reagent for determination of reducing sugar. *Anal Chem*. 1959;31:426–8.
8. Willey JM, Sherwood LM, Woolverton CJ. Microbial Nutrition, Prescott's Microbiology. 8th ed.; 2011.
9. Vijay B. Indoor method of compost production for white button mushroom. *Mushroom Res*. 2006;15:23–7.

10. Sagar A, Kumar K. Study on Ultrastructure and Antibacterial Activity of Ganoderma lucidum (Curt.) P. Kart. *Indian J Mushrooms*. 2012;30:5–9.

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