A Review: Bacterial Diversity, Physicochemical Factors and Quality of Compost for White Button Mushroom Cultivation

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Abstract

Composting is an aerobic biotic decomposition route that is governed by physicochemical and microbiological factors. The importance of bacterial flora throughout composting is well recognized. Though, diversity of the bacterial population during composting may differ with the composting material and nutrients content. Therefore, it is essential to study the different types of bacteria during the composting of various agricultural byproducts. The aim of this review is to evaluate the diversity of bacteria, physicochemical factors and quality of compost for cultivation of *Agaricus bisporus* (white button mushroom) for enhancement of better productivity.

Key words: bacteria, compost, quality, white button mushroom

INTRODUCTION

The mushrooms are spore bearing organisms, lack chlorophyll, and grow on decomposing matter as saprophytes. They derive nutrients through their mycelia. The white button mushroom (*Agaricus bisporus*) is best known in the world. It can be cultivated under favorable conditions. It is cultivated in North India in winter season. The ideal temperature for mycelial development is 22 - 28°C and that for fruit body development 15 - 18°C. The substrate for cultivation is explicitly prepared compost (Pathak *et al*., 2013, Salmones *et al*., 2018).

Presently, more than thirty five mushrooms species have been commercially cultivated. The worldwide production of cultivated edible mushrooms was 495.127 metric tons in 1961. From 1961 to 2019, mushroom production increased to 456901 metric tons (source: UN com trade). In spite of the fact that *Agaricus bisporus* (button mushroom) still holds the most demand in world, now days China is global leader in cultivating mushroom.

The nutritional value of edible mushroom and the bioactive compounds they contain, make mushrooms a healthy food (Andrade *et al*., 2014). Medicinal significance and dietary nature of white button mushroom is well documented (Keleş *et al.,* 2011; Changizi *et al*., 2012; Muszynska *et al.,* 2017, Verma *et al.,* 2013).

Composting is a process of transforming organic materials of either plant or animal origin into simpler compounds (Scialabba *et al*., 2017). It is an aerobic process, during which organic material is biologically decomposed by microorganisms (Jurak *et al*., 2015). The end product should not contain pathogens or viable seeds, and it should be stable and suitable for use. Many factors such as moisture, organic matter, carbon content, EC, pH, C:N ratio, and microorganism, temperature,

affect the composting process and ultimately the end product (Gebeyehu and Kibret, 2013; Jusoh *et al*., 2013; Lim *et al*., 2013).

The problem is that tons of biodegradable organic waste is being generated from agriculture like parali, sugarcane straw, maize straw, wheat straw in the country, creating disposal problems. Through composting mediated by microorganisms that utilize organic waste, the organic matter can become useful in agriculture / horticulture. Composting is one of the most promising technologies to treat waste in a more economical way. For many centuries composting has been used as a means of recycling organic matter back into the soil to improve soil structure and fertility. Composting is a natural process that turns organic material into a nutrient-rich substrate. This is a wonderful conditioner for soil. During composting process, microorganisms such as bacteria and fungi, breakdown complex organic compounds into simpler forms and release nutrients. The turning operation mixes the composting materials, enhances passive aeration and makes conditions for aerobic decomposition. Mixing of substrates has a very important role in composting. During composting some bacteria produce enzymes that breakdown the cell wall elements of the plant's organic material. This cause parts of organic material to start rotting. Some bacteria also release some toxins during decomposition. Careful and continuous monitoring of microbial activity during composting is the key factor of successful decomposition. This can be done through regular turning and mixing of substrates (Navarro *et al*., 2014).

1. Composting

Composting is an environmentally acceptable method that turns agro-waste and organic residues of animal origin into suitable materials for re-utilization. It is an aerobic biological process which uses naturally occurring microorganisms to convert biodegradable organic matter into humus like substance. The process destroys pathogens, converts N from unstable ammonia to stable forms, reduces the volume of waste and improves the nature of the waste. The effectiveness of the composting process is influenced by factors such as temperature, oxygen supply (i.e. aeration) and moisture content. Composting is the decomposition of organic wastes in the presence of oxygen; produce from this process includes CO_2, NH_3, water and heat. Effective composting require right blend of ingredients and conditions. These include moisture content of around 65-70% and C:N ratio 23.18 (Sinha *et al*., 2020, Sarkar and Chanda, 2016; Sofi *et al*., 2016).

2. Phases of Composting

According to Singh and Jha, the four phases of composting include: [1]. Mesophilic phase; [2]. Thermophilic phase; [3]. Cooling phase and [4]. Maturing or Curing phase (Singh and Jha, 2014).

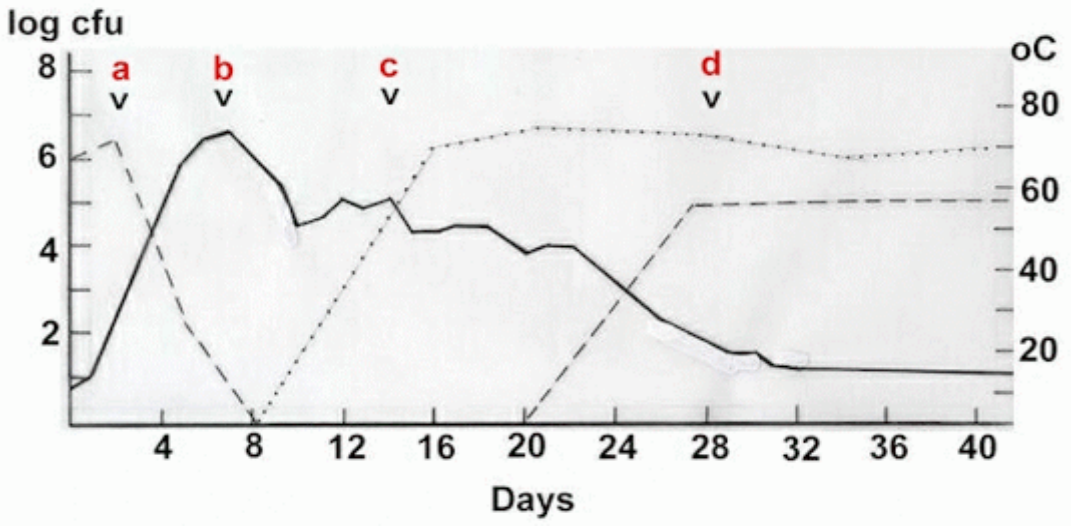

Figure 1. Changes in temperature (solid line) and populations of mesophilic fungi (broken line) and thermophilic fungi (dotted line) in wheat straw compost (Source: http://archive.bio.ed.ac.uk/jdeacon/microbes/thermo.htm).

[i]. Mesophilic phase

In this stage compost bacteria combine carbon with oxygen to produce CO_2 and energy. Some of the energy is used by the microorganisms for reproduction and growth, the rest is given off as heat. When mesophilic bacteria proliferate, the temperature of the composting mass is raising up to 44°C. This is the first stage of composting process. These mesophilic bacteria can include *E. coli* and other bacteria from the human intestinal tract, but these soon become increasingly inhibited by the temperature, as the thermophilic bacteria take over in the transition range of 44-52°C (Gaur *et al*., 2017).

[ii]. Thermophilic (Heating) Phase

During this phase, the temperature in the heap rises to 60-70°C. Most of the decomposition occurs during the heating phase. In this phase, it is mainly bacteria which are active. The high temperature is a result of energy released during conversion of easily decomposable ingredients by the bacteria. The warm temperature is a typical and important part of the composting process. The heat destroys diseases, pest, weed roots and seeds. During this phase of composting process, the bacteria have a very high oxygen demand due to the rapid development of their population. High temperatures in the heap signed that there is an adequate supply of oxygen for the bacteria. If there is not enough air in the heap, bacterial development will be hindered and the compost will develop an unpleasant odour. Humidity is very essential to the composting process, as bacteria require humid conditions for their activities. The need for water is greatest during the heating phase because of high biological activity and strong evaporation occurring during this phase. As the heat increases, the pH of the compost heap rises (Teshome, 2017).

[iii]. Cooling Phase

Once the materials are digested by the bacteria, the temperature in the compost heap declines slowly and remains at 25-45°C. The decline in temperature, fungi settle and start the decomposition of straw, fibers and other organic material. As the temperature drops, the composting material declines (i. e. acidity increases) (Teshome, 2017).

[iv]. Maturity Phase

During the maturing phase mineralized nutrients, humic acids and antibiotics buildup. At the end of this phase the compost has lost about half of its original volume, is the dark in color, fertile and ready to use. In the maturity phase, the compost needs much less water than in the heating phase (Eiri, 2018).

3. Factors affecting the composting process

The breakdown of organic matter during composting is a constantly change in moisture, oxygen demand, nutrients, temperature, and pH (Teshome, 2017).

[i]. Moisture

At too high temperature water between the particles of material gets waterlogged, preventing movement of air within the heap. The optimum moisture content of ingredients for composting is 50-60%. Water is produced during the composting process by the microorganisms and is lost by evaporation into the air stream. It may be necessary therefore to provide additional moisture to the compost heap (Singh and Nain, 2014).

[ii]. Oxygen demand

An adequate supply of oxygen to all parts of a compost heap is essential and flush out the carbon dioxide produced. Oxygen supply to heap by turning the material and flush out the CO_2 (Andrade *et al*., 2013).

[iii]. Nutrients

The composting process depends upon the activity of microorganisms which require a source of carbon to provide energy and material for new cells, together with a supply of nitrogen for cell proteins. Nitrogen is the most important nutrient. It is desirable that the ratio of carbon to nitrogen is in the range of 25 to 35/1. The simplest method of adjusting the C/N ratio is to mix together different materials of high and low carbon and nitrogen contents. Phosphorus is less important nutrient in composting than nitrogen but sometimes deliberately added (Gaur *et al*., 2017).

[iv]. Temperature

When organic material is mixed for composting some of the energy released by the breakdown of the material causes a rise in temperature. The normal temperature-time curve of a compost heap is shown in the fig. 2 (Smith and Collins, 2007).

When the composting material passed through the temperature peak, the heap reaches stability at which the easily converted materials broken down and it is met the highest level of oxygen demand. While cooling down, straws and stalks get decomposed; hence hemicellulose and cellulose break down, into simpler sugar components, for the microorganisms. It is necessary to kill all

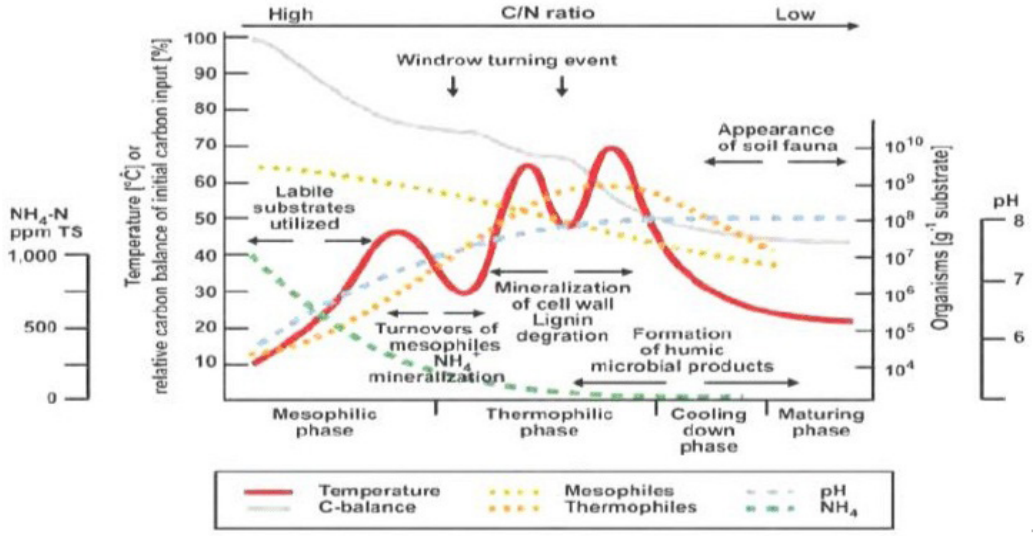

Figure 2. During Composting process varied Temperature, pH, NH_4 and Carbon composting (Lechner *et al.*, 2005)

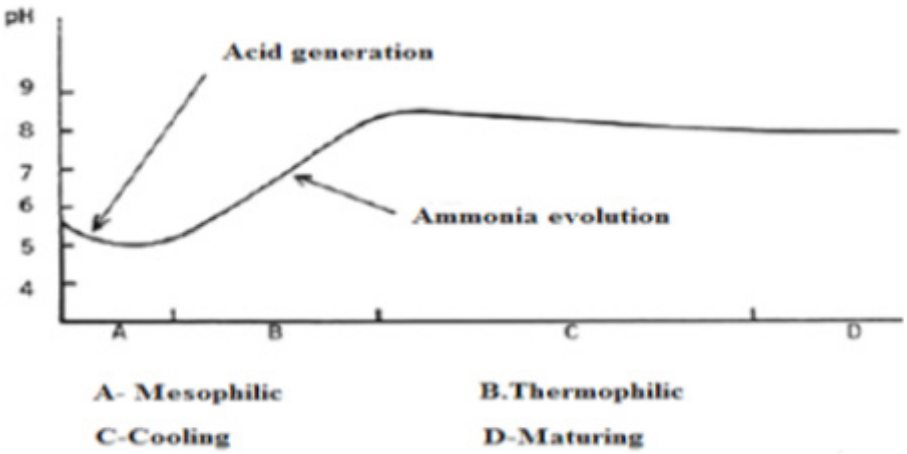

Figure 3. The pH variation in a compost heap (Dalzell *et al.*, 1987)

weeds and disease-causing by microorganisms (Singh and Jha, 2014).

[v]. pH

The composting pH depends on the source materials and varies in each phase of the process (from 4.5 to 8.5). In the early phases of the process, the pH was acidified by the formation of organic acids. In the thermophilic phase, due to the conversion of ammonium into ammonia, the pH rises; the medium is alkalized to finally stabilize at values close to neutral. The pH determines the survival of microorganisms and each group has optimal pH for growth and multiplication. Most bacterial activity occurs at pH 6.0 - 7.5, while most fungal activity occurs at pH 5.5 to 8.0. The ideal range is from 5.8 to 7.2. (see fig. 3) (Dalzell *et al.*, 1987).

4. Composting Methods and Turning

Turning means aerating of the compost pile. The materials in a compost heap are the deciding factors whether composting is an acceptable practice in a community. It helps mix the various ingredients in the heap. It aids aeration where air has difficulty in penetrating to the middle of the heap. It also gives an opportunity to moisten the material. Due to turning the mesophilic, thermophilic

and cooling down stages take place in about 7-10 days (Singh *et al*., 2011). There are two methods of composting adopted for *Agaricus bisporus* production: LMCT (Long Method of Composting and Turning) and SMCT (Short Method of Composting and Turning) (Singh *et al*., 2011).

[i]. LMCT (Long Method Composting & Turning)

This method usually takes 26-28 days. On first day, wheat straw and paddy straw are mixed with fertilizer and bran measuring heap size 6 feet × 3 feet × 4 feet.

First turning of compost gets carried out on 6th day with addition of 5 kg molasses and heap is formed again. The second turning is done on the 10th day and 150 g furadan 3G (Carbofuran) is mixed. Third turning is given on 13th day and mixed with required quantity of gypsum. Likewise, the fourth, fifth, sixth turning done on 16th, 19th and 22nd day respectively. On 25th day, 25g BHC – Benzene Hexa Chloride, 10% is added during seventh turning. Filling of compost is done on 28th day. If there is smell of ammonia, then it is turned and kept open for one to two days more. However the excess ammonia will disappear and the compost will be ready for further use. Compost preparation is very important process for cultivation (Andrade *et al*., 2013). Various types of formula are used for composting and used by organizations such as ICAR, NCMRT, IIHR, and CCSHAU(Choudhary Charan Singh Haryana Agricultural University) (Singh and Jha, 2014).

[ii]. SMCT (Short method of Composting and Turning)

In this method all ingredients are mixed as per LMCT. First turning is given on 2nd day. Similarly 2nd turning on 4th day and 3rd turning on 6th day. However, on 8th day of compost transfer for pasteurization and heating.

This method is comparatively better than LMCT due to several aspects: compost preparations take 14-18 days where as LMCT take 26-28 days; production of mushroom per unit compost is almost double; manpower requirement is also less as compared to the LMCT.

The method is carried out into two steps: first pasteurization and second heating (Sinha *et al*., 2020).

Pasteurization

Mixed ingredients of compost on 8th day transferred into tunnel/room. The size of tunnel or insulated room is 36 ft. × 9 ft. × 12 ft. for a capacity to produce 20-22 tons. A boiler capacity of 15 kg / circulates steam with a capacity of 145 rpm to produce 150-200 m^3 air / h. (Eiris, 2018).

Heating

Pasteurized compost on 9th day is transferred into heating room. In heating room compost is placed into number of 250 trays with tray size 100 cm × 50 cm × 15 cm, room size 24 feet × 16 feet × 8 feet on the stand of 15 cm long. About 12-15 h of compost filling in chamber the temperature of compost starts rising and once reached up to 48-50°C. This temperature is maintained for 36-40 hours. With self-generation of heat of the compost mass without steam injection. Thereafter temperature of compost rises up to 57°C by injecting the steam. Temperature is maintained for 6-8 hours. During 10th to 14th days temperature is gradually lowered down to 45-50°C till no traces of ammonia get detected in the compost. This may take 3-4 days. On 15th-18th day, when the compost is free from ammonia, full fresh air is introduced, the compost temperature cools down to 25°C and is ready for mushroom cultivation. Various compost formulations can be used for button mushroom cultivation i.e. natural compost, synthetic compost and CCSHAU (Chaudhary Charan Singh Haryana Agricultural University) (Andrade *et al*., 2013; Borkar and Nisha, 2016; Singh and Jha, 2014).

5. Bacteria Associated with the Compost

High concentration of organic matter contains many micro fungi and bacteria. However most parasitic organisms are killed during heating phase of compost, a few spore –forming bacteria, if present, can survive temperature more than 100°C. The composting material like coastcross straw, *Saccharum officinarum* bagasse and other nutrients are used for farming of *Agaricus brashiliensis*. Total composting period lasts for 14 days followed by two-step steam pasteurization at 55-65°C for 15 h. Results showed that mainly *Bacillus* and *Paenibacillus* spp. and the members of *Entrobacteriaceae* with average population density of 3×10^{8}CFU/g are present. Similarly, *Actinomycetes, Streptomyces* populations reach between 2-3×10^8/CFU/g. The other filamentous fungi population density is found lower. *Aspergillus fumigatus* are detected in compost from first day to fourteen days (Silva *et al*., 2009). Concertation of dehydrogenases determined by 1% triphenyltetrazolium chloride as substra-

tum showed that in all composting process *Entrobacteriaceae* was eliminated. Inactivation of bacteria from *Enterobacteriaceae* family and reduction of *Clostridium perfringens* bacteria testify to high level of hygienic effectiveness of compost processing (Wolna and Sawicka, 2009). Bacterial identification can follow the specific standard method (Bull *et al.,* 2010*;* Bull *et al.,* 2012*)*. The growth of denitrifying atmosphere is very favorable to denitrifying bacteria. *P. denitrificans* could denitrify nitrite when their concentrations were lower (10 mM). A toxic outcome of nitrite at high concentration dropped the growth of *Pseudomonas denitrificans* and other denitrifies. *P. denitrificans* first decrease nitrate to nitrite under denitrifying conditions and then, after reducing most of the nitrate, initiates the subsequent reduction from nitrite to the nitrate and subsequent reduction from nitrite to N_2 (Miyahara *et al*., 2010).

Bacterial profile in casing materials are used for *Agaricus bisporus* cultivation. The casing materials were collected from mushrooms growing farm. In casing materials are used wattle bark, coir, filter cake, bagasse and lime added to stabilize the pH from acidic to pH 7. 0. The methodology was used for DGGE (Denaturing Gradient Gel Electrophoresis) analysis for bacterial characterization. The bacteriological study was conducted in three different stages: first casing, second pinning and third harvesting. Results showed that bacterial population were countless in the peat based mixture, which contained industrial discarded materials, than in peat alone. Results showed that numerous types of the bacterial population identified in peat casing materials and as potential bacterial like *Flavobacterium, alpha-Proteobacterium, Pseudomonas, gamma-Proteobacterium, beta-Proteobacterium, and delta-Proteobacterium* community supported in the pinhead initiation process (Siyoum and Korsten, 2010).

Choudhary (2011) studies in casing soil of *Agaricus bisporus* showed that *Pseudomonas* and *Acinetobacter* of the *γ-proteobacteria* were the maximum encountered species. Other several genera and species contained are *Pseudomonas mevalonii, B. subtilis, Bacillus licheniformis* and *Paenibacillus lentimorbus.* Another two microbial flora *Stenotrophomonas* sp. and *Sphingobacterium multivorum* were also examined. In addition two potentially novel types within the genera *Microbacterium & Stenotrophomonas* and various dynamics of microorganism was found during maturation phase of composting (Choudhary, 2011; Villar *et al*., 2016).

Button mushroom has most important economic value, medicinal characteristics and is rich in nutrition. Wheat straw and sugar cane bagasse are selected for compost. Results stated that a diverse population consisting mainly of *Bacillus* sp., followed by *Ochrobactrum* sp., *Arthrobacter arilaiti*, *Stenotrophomonas maltophilia* are responsible for the degradation of the physical, chemical and fibers characteristics of the compost (Johri, 2011).

Discoloration of *Agaricus bisporus* (white button mushroom) due to pathogenic bacteria was reported by Abou-Zeid (2012). Superficial brown discolored caps of button mushroom collected and from that 27 bacterial isolates were identified. Bacterial isolate were identified with Biolog identification system and WLA (White Line Assay) and was divided into two groups. First group contained 19 bacterial isolates which responded to *Pseudomonas reactans* and *Pseudomonas tolaasii.* These two bacteria belong to strain NCPPB1311. Second group belongs to strain JCM21583. Third group comprises 6 bacterial isolates. In this group first subgroup contained *Pseudomonas tolaasii* and *Pseudomonas gingeri* and in second four isolates of *Pseudomonas fluorescens* (three strains) and *Pseudomonas marginalis* (one). First treatment with 10 µl containing 108 CFU/ml^{-1} of *P. tolaasii* and *P. reactans* showed light brown color and 50 µl containing 108 CFU/ml^{-1} of *P. tolaasii* significantly visible typical brown blotch indicator on the fresh mushroom sporophores whereas *P. reactians* showed superficial brown discoloration after inoculation with 100 µl of same concentration. This study indicates that mixture of both bacterial suspensions increased the discoloration on the pileus. Research article stated that this is the first pathogenicity test carried out on the *Agaricus bisporus* for pathogenicity with *P. tolasii* and *P. reactans*. Some of the significant nitrogen fixing bacteria beneficial for agriculture are from genera: *Achromobactor, Acetobacter, Alcaligenes, Arthrobacter, Azospirillum, Azomonas, Beijerinckia, Azotobacter, Clostridium, Corynebacterium, Bacillus, Derxia, Herbaspirillum, Klebsiella, Enterobacter, Pseudomonas, Rhodospirillum, Xanthobacter, Rhodopseudomonas*. Some of

other phosphate solubilizing bacteria like *Bacillus cereus, B. polymyxa*, *B. pumulis, Pseudomonas fluorescens, P. aeruginosa* play very important role in the soil for release the phosphate. According to other studies it was stated that lignin degrading enzymes like lignin peroxidase, manganese peroxidase, glyoxal oxidase are present (Girisham *et al*., 2012).

Swain *et. al* (2012) conducted a study on the *Bacillus subtilis* from cow dung to investigate the role in phosphate solubilization. *Bacillus subtilis* grows at <50°C and are known as thermo-tolerant bacteria. Results showed that *Bacillus subtilis* successfully solubilized phosphorus in soil. Therefore, its very useful bacteria for agriculture (Stanojevic *et al.,* 2016*;* Swain *et al*., 2012).

Chung *et al*. (2014) conducted studies on *Pseudomonas tolassii* in *Agaricus* to identify pathogenic characteristics. The major decreasing quality of mushroom are due to infections of mushrooms and it can be improved by various washing treatment (Gupta and Bhat, 2016). *Pseudomonas tolaasii* produces peptide toxin known as tolaasin. In total seven strain of *Pseudomonas tolaasii* were isolated from *Agaricus bisporus* (Chung *et al*., 2014).

The microbiological additive effects on composting was studied by Lim *et al*. (2013). In this study various factors like C:N ratio, temperature, moisture, pH, bacteria, fungi, and *Actinomicetes* were studied. The inoculation of microbes in compost increase soil fertility and crop production. Research article showed that different bacteria from compost like *Pseudomonas, Cohnella, Cellulomonas, Paenibacillus, Bacillus, B. licheniformis, B. subtilis, B. coagulans, B. stearothermophilus, B. sphaericus*, *Thermotogae* and fungi like *Aspergillus fumigatus, Malbranchea cinnamomea, Ganoderma colossum* and *Heterobasidion annosum* were present in various composts. In addition of additives the use of compost also suppress diseases and increase plant stress acceptance capacity. Use of compost as organic manure enhances soil fertility and reduced production of toxins in the soil (Lim *et al*., 2013; Reddy *et al*., 2017).

The odour in compost plays vital role and various impacts on pH and microbial composition. During composting, specific odor occurs due to low decomposition process. *Actinobacteria (Corynebacterium), Bacillales (Bacillus infernus, B. thermoamylovorans), Lactobacillus* species *(L. amylovorus, L. brevis, L. plantarum)* were found in compost. Other genera and species like *Bacillus pumilus, Thermoactinomyces, T. chromogena, T. curvata* and *Thermobifida fusca* (*Thermomonospora*) were also detected. During investigation it was noticed that pH ranges from 6.5 to 6.0 while nitrate, TVOC (Total Volatile Organic Compounds) and organic acids were not detected. Spent mushroom substrate are also useful to incorporate in white button mushroom production. Results finally concluded that reduction in odour, stable pH aeration and additives are usually required (Rossiana *et al*., 2017; Sundberg *et al*., 2013).

Composting is the biological degradation and stabilization of an organic substrate under the conditions that permit growth of thermophilic microorganisms and that generates heat as a natural outcome. Cellulolytic bacteria degrade cellulose. Others bacterial strains are well-known to solubilize and change the lignocellulosic structure extensively. Mesophilic bacteria *Cytophaga* and *Cellulomonas* are known to degrade cellulose. Other hemicellulose and cellulose degrader mesophilic bacterial species are *Bacillus megaterium, Bacillus polymyxa, Bacillus brevis, Bacillus firmus, Bacillus cerus, Bacillus licheniformis, Bacillus circulans, Bacillus subtilis* and *Bacillus pumilus* (Singh and Nain, 2014).

Growth promoting and anti–microbial activity of bacteria from fruit body of *Agaricus bisporus* has a very significant role in environmental development of cultivated mushrooms worldwide. According to Zheng *et al*. (2018) mushroom growth-promoting microorganism are isolated from earth soil and compost. A total of 55 microorganisms were isolated and categorized from *A. bisporus*. Around 9 strains of *Actinomycetes* were identified while other microorganisms were analyzed by 16S rRNA and 16S rRNA factor sequence. No less than 11 isolated groups were gram positive true bacteria like *Lysinibacillus, Paenibacillus, Pandorea* genera while other five microorganism taxa were gram-negative bacteria. Some bacteria identified are involved in several biological processes including the transformation, mineralization and solubilization of soil phosphate. Trial materials were collected from the farming fields and tested in the laboratory by phylogenetic identification with 16SrRNA and PVK (Pikovaskayas) medium. Results showed that four iPSB strains with the

highest phosphate solubilizing capacity were representative. It was showed that *Bacillus megaterium* phosphate solublization capacity is higher than other genera including *Streptomyces, Arthrobacter* and *Pseudomonas*. The *Bacillus megaterium* isolates Y1412, Y95, Y99 and Y924 released more than 130μg/mL^{-1}. Succinic acid concentration were powerfully and linearly correlated with phosphate release (R^2=0. 7908; P value of, 0. 001) (Zheng *et al*., 2018).

Compost management design has direct potential impact on the mushroom production, control of *Lycoriella ingenua* larvae and the environment (Cloonan *et al*., 2016; Jayaprakash *et al*., 2018).

It was also stated that compost is very important process to recycle nutrients like nitrogen and phosphorus. Methods of microbial analysis of compost and investigations about the relationship between the indigenous microorganisms, foodborne pathogens, C:N ratio, moisture and temperature are all needed to ensure that compost can be used as a biological soil amendment to grow fruits and vegetables intended for human consumption (Gurtler *et al*., 2018).

6. Compost Quality

The intent and need to report qualities of compost scientifically is a natural outcome of growth of the compost industry. The concept of establishing quality standards specific to compost is a very important factor. Recently, several European countries have adopted specific standards and many other countries are in the process. The only existing quality guidelines specific to compost are presently promulgated by such specific agencies. However, compost, a product that contains nutrients and organic matter, is not subject to any systematic rules for reporting its content, qualities or potential risks. There are no labelling rules and no published guidelines to establish such rules, unless if the compost is sold as fertilizer. Following quality composition of compost permissible limits are accepted by various countries (Table1, Table 2, Table 3, Table 4).

7. Cultivation of White Button Mushroom and Bacteria

The production of *Agaricus bisporus* depends on spores and spawn. When the spores of the mushroom fungus fall on the suitable substrate and the conditions are favorable, they germinate and form a mat of mycelium. This is dug out and use as spawn. Second type of spawn is "Flake Spawn" when the beds fully covered with mycelium before a crop of mushroom appears. Then the compost is collected, broken, dried and used as fresh to inoculate other new bed. Third type of spawn known as "Brick Spawn" when a mass consisting of horse and cow dung manure and loam is mixed with water, tapped out into layer two inches thick and cut into pieces when it half dry. These pieces are then inoculated with old spawn by making a hole in each and after the spawn grows through the entire piece it is dried and sold as Brick Spawn. Fourth type of spawn is known as "Grain Spawn" and is suitable and worldwide accepted for the preparation *Agaricus bisporus* spawn. The grain is boiled with equal volume of water till the water dries out. Then grain mixed with calcium carbonate, 8% by the grain weight. The mixed grain is filled in a wide mounted bottle, plugged tightly and sterilized for two consecutive days at 15 lbs p.s.i for 30 minutes. After two days of sterilization the bottle inoculated with the culture of mushroom at 25±1°C for 21 days. By combining 2% gypsum with 6% lime by grain weight gives the best results in the process of making grain spawn for *Agaricus bisporus* (Bahl, 2018).

Prepared spawn is stored at 2°C for 68, 128 and 206 days. The compost is filled in wooden trays of convenient size having four pegs on four corners or it can be put on shelves. Spawn trays are covered with paper and stacked. Room temperature should be maintained between 22-25°C during spawn run. Compost is covered with a thin layer of soil after 10-15 days. Most suitable thickness of the casing is 1-1.5 inches for button mushroom production. After casing, the mushroom can be expected between 5-20 days. A fine water spray is given to casing soil to maintain moisture 70-80% of humidity. Room temperature should be kept between 14-18°C for better yield. Pin head formation happens after 7-8 days. Hence an interval of 8-10 days mushroom occurs between flushes. The maximum mushroom production can be considered at 6 week in order to be economically viable (Singh *et al*., 2011).

Bacterial blotch diseases is one of the most common in *Agaricus bisporus* and is caused by *Pseudomonas tolaassi*. *Agaricus bisporus* cap sunken spots are irregular and colour becomes yellowish to dark brown. The main source of infection is the casing soil. Preventive spray with 9

Table 1. Nutrients limit in compost for mushroom cultivation (Roman *et al.*,2015)

No	Nutrients	% (g/kg)
1	Nitrogen	0.3 - 1.5 (3g - 15g/kg of compost)
2	Phosphorus	0.1 - 1.0 (1g - 10g/kg of compost)
3	Potassium	0.3 - 1.0 (3g - 10g/kg of compost)

Table 2. Microbiological limits according to different standards (Brinton, 2000)

Microorganism	Chile Nch 2008/04		EU (European Union)	Colombia 5167/04	Mexico NTEA-006-SMA RS2006
	A	B			
Fecal Coliform (Dry base	<1000g NMP/g	<2000 NMP/g	$<1\times10^3$ NMP/g	<1000UFC/g Total Entro bacterial	<1000 NMP/g
Salmonella sp	Absent in 25g of product	Absent in 25g of product	Absent in 25g of product	Absent in 25g of product	<3/gbs
Enterococcus faecalis	_	_	1000 NMP/g	ND	_
Visible helminth eggs/Ascaris	Absent in 1g	Upto 1 in 1g	Absent in 1g	ND	<10/g bs

Note: NMP-Most Probable Number, UFE-Colony formation units, bs- Dry base

Table 3. Heavy Metals limits (mg/kg) compared EC State verses USA (Brinton, 2000)

Metal	Symbol	EU-Range	USA bio solids
Cadmium	Cd	0.7-10	39
Chromium	Cr	70-200	1200
Copper	Cu	70-600	1500
Mercury	Hg	0.7-10	17
Nickel	Ni	20-200	420
Lead	Pb	70-1000	300
Zinc	Zn	210-4000	2800

Table 4. General factors limit for compost (Gaur *et al*. 2017)

Condition	Preferred range
Moisture %	68-72
C:N ratio	17.6-20.47
Oxygen %	>10
pH	6.5-8.0
Particle size	1/8-2 inches
Dry Matter	5.5-8.0%
P	0.35-0.41%

mg/ft. of tetracycline or Bavistin 250 mL/100 m^2 on the beds are quite effective (Eiri, 2018; Bahal, 2008).

CONCLUSION

Button mushroom is a delicious fungi and considered worldwide as healthy part of the human diet. Mushrooms have been used as food source since ancient times. Cultivation of edible mushrooms has significantly increased across America, Europe, and Asian countries. White button mushroom contains a wide range of nutritional components: ash (7.01 - 17.92%), fibers (15.42 - 29.02%), proteins (18.31 - 41.06%), fats (1.54 - 5.38) and carbohydrates (28.38 - 34.88%).

Agaricus bisporus is cultivated on compost containing organic nutrients and bacterial load. During the cultivation bacteria affects the yield, quality and nutritive value of button mushroom. Presently various agricultural wastes such as parali, sugar cane straw, paddy straw could be used for mushroom cultivation.

Bacteria has a vital role in composting and white button mushroom production. This review concluded that various types of bacteria have various roles in composting. Some of them could be harmful while others are useful. Detection of bacteria associated with turning needs future studies to understand their role in decomposing, mineralization and quality production of *Agaricus bisporus*.

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