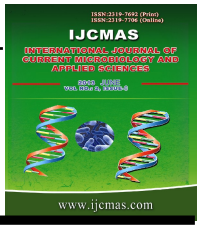
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 2 Number 6 (2013) pp. 162-175**
http://www.ijcmas.com



Original Research Article

Functional and genetic characterization of culturable bacteria associated with late phase of mushroom composting assessed by amplified rDNA restriction analysis

Abhinay Sharma[1], Ajay Veer Singh[2*] and Bhavdish N. Johri[3]

[1]Department of medical microbiology, Helmholtz center for infection research, Inhofenstrasse, 7, Braunschweig, Germany
[2]Department of Microbiology, College of Basic Sciences & Humanities, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar 263145, India.
[3]Department of Biotechnology, Barkatullah University, Bhopal 462 026, India
***Corresponding author e-mail:** ajaygbpuat@gmail.com*

A B S T R A C T

The edible mushroom *Agaricus bisporus* is grown commercially on composted manure/straw mixtures. Mushroom composting is a fermentation process in which various groups of microorganisms play important role at different stages of composting. Present study was conducted to explore the mesophilic culturable bacterial diversity in late phases of mushroom composting. Morphologically all the isolated bacteria were gram positive rods. The functional diversity of the bacterial isolates was examined by plate enzyme assays, siderophore production, antagonistic property and quantitative enzyme assays. Good enzymatic activity for amylase, cellulase, xylanase and protease was reported for different bacterial isolates. Fifteen bacterial isolates showed siderophore production activity. During antibiosis assay most of the isolates inhibited growth of *Verticillium fungicola* but not of *Mycogone perniciosa.* However, Consortium of selected bacterial isolates produced good amounts of lytic enzymes (amylase, cellulase, xylanase and protease) in solid state fermentation experiment that might help in enhancing composting process. Amplified 16S-rDNA restriction analysis (ARDRA) showed that only two bacterial isolates (JCA and KCA) were 100% similar in all three restriction profiles (Sau 3AI, Msp I and Taq I) and two isolates (FC and HC) were 100% similar in two restriction profiles (Sau 3AI and Taq I). However, other isolates exhibited discriminatory relationship with each other when analyzed for combinations of restriction profiling. The present study reveals bacterial diversity and community succession in late phase of composting process as well as emphasizes on application of bacterial consortium to enhance composting process.

Keywords

Mushroom composting; *Agaricus bisporus;* ARDRA; 16S rDNA; Phylogenetic diversity

Introduction

White button mushroom Imbatch (*Agaricus bisporus*) is the most widely cultivated and popular species of edible mushroom among the artificially grown

fungi of the world that contributes about 31.8% of the global mushroom cultivation and 85% of the total production in India. Composting is an advantageous biotechnological process to recycle lingo-cellulosic waste which can be applied to produce protein rich mushrooms for human consumption and spent mushroom substrate can be utilized profitably in different ways (Masaphy *et al*., 1987).

Agaricus bisporus is cultivated on a substrate consisting of a composted mixture of straw bedded horse manure, wheat straw, chicken manure and gypsum. Conventially two phases of mushroom composting are distinguished (Fermor and MaCauley, 1991). After mixing and moistening these ingredients are subjected to a phase I composting process. Mixed ingredients are stacked in windrow in the open air for uncontrolled self heating (up to 80°C) for 1 to 2 weeks. Phase II is an aerobic process carried out by maintaining the compost at 40-58°C for 6 days in shallow layers in mushroom houses. The decomposed straw thus becomes a source of organic and inorganic nutrition for mushroom mycelium (Wood and Fermor, 1981). *Agaricus bisporus* is seeded in prepared compost as spawn (Straatsma *et al*., 1994). The compost is colonized by mushroom mycelium in 2 weeks time at 22°C, where upon it is covered with casing material which is usually inert, low in nutrients with high water holding capacity, and pH of 6.0-8.5.

Compost preparation is the result of microbial community succession. Microbial community changes result in changes in compost in such a fashion that favors the growth of another microbial community. Detailed succession of microbes on compost has been studied and a large number of microorganisms associated with the composting process have been isolated and characterized (Beffa *et al*., 1996; Blanc *et al*., 1997). In present study, we investigated the morphological, functional characterization and phylogenetic relationship of the culturable mesophilic bacterial population associated with late phase of mushroom composting. Our findings of this work might be helpful for the monitoring of phylogenetic profiling of culturable bacterial diversity and community succession in late phase of mushroom composting. Further we concluded that the potential microbial inoculants might be beneficially used to improve the conditions for composting, spawn run in the substrate and in disease management.

Materials and Methods

Isolation of bacterial isolates

Twenty bacterial isolates were used in this study, which belonged to different stages of phase II mushroom composting and originally isolated from Mushroom Research and Training Centre (MRTC), and collected from the department of Microbiology culture collection, College of Basic Sciences and Humanities, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India. The compost samples were collected axenically from four different stages (airing, drenching, spawn run and cropping) of phase II mushroom composting process and casing soil. Dilutions (10^{-6}, 10^{-7}, 10^{-8}) of compost samples were plated on nutrient agar (NA: beef extract, 3 g; peptone, 5 g; agar agar, 20 g; distilled water, 1000 ml; pH, 7.0±2) medium. Three plates of nutrient agar medium were kept for each dilution and incubated at 28±2°C for 3 days in BOD incubator. Morphologically distinct and

isolated colonies were transferred to respective media and purified thereafter following standard protocol (Holt *et al.*, 1994; Silva *et al.*, 2009). All the bacterial isolates were maintained on nutrient agar slants at 4°C for regular use and in 15% glycerol stocks for long time preservation at -80°C.

Morphological characterization

Colony morphology of the isolates was studied under a stereoscope microscope (Olympus, SZH 10). This included shape, edge, elevation, surface and chromogenicity. However, the cellular morphology was based upon cell shape and Gram staining (Leica fluorescent microscope).

Functional characterization

Assessment of functional diversity among the isolates was carried out by qualitative plate assay for amylolytic, proteolytic, cellulolytic, xylanolytic activity and siderophore production. Minimal medium was used for plate assay, wherein isolates were spot-inoculated with sterile tooth-pick on solid medium and incubated at 28±2°C. The diameter of zone of clearing, if any, for all positive isolates was measured.

Amylase (Starch diastase)

All the overnight grown bacterial cultures were spot inoculated on amylase producing minimal medium amended with 1% (w/v) starch and incubated for 24-72 h at 28±2°C for growth. Plates were flooded with Lugol's iodine for 10 minute. Then iodine was drained off and positive isolates exhibited a zone of clearance against dark blue background.

Protease

The protease producing medium amended with skimmed milk (20 ml L^{-1}) was spot inoculated with overnight grown bacterial isolates. Plates were incubated for 24-72 h at 28±2°C for growth. Formation of clear halo zone around the bacterial colony was considered as positive result for this test.

Cellulase and xylanase

The minimal medium was supplemented with 1% (w/v) birch wood xylan and carboxymethyl cellulose (CMC) and spot inoculated with bacterial isolates. Plates were incubated for 72 h at 28±2°C for growth and flooded Congo red solution (0.2% w/v) for 30 min. Excess reagent was discarded after destaining with 1M NaCl solution for 30 min. Zone of clearance around bacterial colonies was considered as positive result.

Siderophore Production

Chromeazurol 'S' agar plates were spot inoculated with bacterial isolates, and incubated for 48-72 h at 28±2°C for growth. Formation of an orange halo around bacterial colony was considered as positive result (Schwyn and Neilands, 1987).

Solid state fermentation

For the solid state fermentation, nine potential bacterial isolates were selected on the basis of qualitative enzymes assays and the mixture of the isolates were inoculated on '0' day mushroom compost as substrate. The 20 g of substrate was dispended in 250 ml flask and 50 ml of sterilized distilled water was added to achieve a moisture level of 2 ml g^{-1} solid. The experiment was carried out in two sets

of treatments, I set consisted of inoculated autoclaved substrate and un-inoculated autoclaved substrate as a control. However, II set consisted of inoculated un-autoclaved substrate and un-inoculated un-autoclaved substrate as a control. Flask were aseptically inoculated with 5 ml (2.1×10^8 cfu ml^{-1}) of mixed bacterial suspension in triplicate and incubated at room temperature for 7 days.

Enzyme assays

After 7 days of incubation 100 ml phosphate buffer (0.1 M, pH 7.0) was added to fermented mixture for enzyme extraction. Flask were placed on a rotary shaker for 1 h at 180 rpm and then left for overnight under refrigeration for the release of any bound enzyme. The contents were centrifuged at 8000 rpm for 15 min and the supernatant was used as crude enzyme.

α-Amylase activity was determined by incubating 1 ml reaction mixture containing 0.1 ml culture filtrate, 0.4 ml 50 mM sodium acetate buffer (pH 5.0) and 0.5 ml of 1 percent starch solution, at 45°C for 5 min. The reaction was terminated by adding 1 ml of 0.5 M HCl; 0.5M iodine reagent (0.25% I_2 + 0.5% KI) was added to develop the colour and absorbance was recorded against reagents as blank at 540 nm Chadha *et al.,* (1997).

Xylanase and cellulase activity were assayed by dinitrosalicylic acid (DNS) method (Miller, 1959) using a calibration curve of D-xylose and D-glucose, respectively. Xylanase activity was determined by incubating 0.5 ml of culture filtrate and 0.5 ml of 1% xylan solution in 0.1 M phosphate buffer (pH 6.0), however the cellulase activity by adding 0.5 ml of culture filtrate and 0.7% carboxy methyl cellulose solution in 0.1 M phosphate buffer (pH 6.0) and both the mixture were incubated at 30°C for 30 min. The reaction was terminated by adding 1 ml of dinitrosalicylic acid (DNS) and boiled for 10 min in a water bath. The contents were cooled at room temperature and then added 400 µl of 33% Na-K tartrate to develop the colour and absorbance was recorded against reagents as blank at 540 nm.

Protease activity was measured following the already published method Upton and Fogarty (1977). Briefly, casein was used as substrate and dissolved (0.5%) in 0.05 M glycine HCl buffer (pH 3.0). For assay, 1 ml casein was added to 1 ml culture filtrate and the reaction mixture incubated at 37°C for 30 min. The reaction was terminated by the addition of 2.0 ml of 0.44 M trichloroacetic acid (TCA). The precipitated protein was filtered through Whatman No.1 filter paper. 1 ml of TCA filtrate was added in 5 ml of 0.44 M Na_2CO_3 followed by 1 ml of Folin's reagent (1: 1 v/v). The liberated protein was estimated according to protein estimation method of Lowry *et al.,* (1951).

Enzyme activity is expressed in terms of International Unit (IU). It is defined as the amylase, xylanase and cellulase enzymes that hydrolyze 1mg of starch, xylose and glucose per minute; however, the protease activity is expressed as 1 mg of tyrosine released by 1 ml of enzyme solution in 30 min. The amount of enzyme activity was read from the standard curve of starch, xylose, glucose and tyrosine, respectively.

Antibiosis assay

All the isolates were tested for antagonism against pathogenic fungi of mushroom, *Verticillium fungicola* and *Mycogone*

perniciosa. The actively grown fungal culture (5 mm disc) was placed in the centre and the bacterial culture spotted towards the periphery of culture plate, which was incubated at 20± 2°C for 7 days. Inhibition of growth of pathogenic fungi around the bacterial colony was considered as positive results.

Genotypic Characterization

Recovery of Genomic DNA

Total DNA from bacterial isolates was prepared following the already published procedure with the exception that for Gram-negative bacteria, no lysozyme was used (Bazzicalupo and Fani, 1994). Extracted genomic DNA was run in 0.8% agarose gel at 80 V for 45 min. DNA was quantified spectrophotometrically by measuring OD at 260 nm and 280 nm. Purity of DNA was checked measuring the extinction at A_{260}/A_{280} on a DU 640 B Beckman spectrophotometer.

PCR Amplification of 16S rDNA

The PCR amplification of 16S rDNA for each of bacterial isolate was performed by using the eubacterial universal primers GM3f (5' AGAGTTTGATCMTGG 3') and GM4r (5' TACCTTGTTACGACTT 3') which were targeted at universally conserved regions and permitted amplification of ~ 1,500-bp fragment (Weisburg *et al.*, 1991). PCR amplification was carried out in a PTC-200 thermocycler (MJ Research). Initial DNA-denaturation and enzyme activation steps were performed at 95°C for 7 min, followed by 25 cycles of denaturation at 94°C for 1 min, annealing at 51°C for 1 min and extension at 72°C for 1 min, and a final extension at 72°C for 10 min.

Amplified 16S rDNA restriction analysis

Amplified 16S rDNA was digested with three restriction endonucleases; *Msp1, Sau 3AI* and *Taq1*. Total of 15 µl (120 µg) of amplified 16S rDNA was digested with each of restriction endonucleases in the reaction mixture. For the preparation of 30 µl reaction mixture, the following were added: Restriction enzyme (10 U), 0.05 U (MBI Fermentas); Restriction buffer (10 X), 1.0 X (MBI Fermentas); 16S rDNA amplicon 120 µg, and Milli Q water. The reaction mixture was incubated at 37°C for *Msp1* and *Sau 3AI*, and at 65°C for *Taq 1* enzyme for 4 h. Restriction product was resolved on 2.5% agarose gel in 1X TBE at 70V for 4 h, the gel was stained with ethidium bromide (0.5 µg ml^{-1}) and visualized on a UV transilluminator (GelDocMega, Biosystematica). The restriction profile was analyzed using NTSYS pc version 2.02i. The clustering was done using Jaccard's similarity coefficient based on presence and absence of band ignoring their intensities.

Results and Discussion

Morphological and Functional characterization

During the study of morphological and functional characterization, considerable and significant differences were observed among the bacterial isolates recovered from the different stages of the phase II mushroom composting (airing, drenching, spawn run and cropping) and casing soil. During this microbial succession, each type of microorganism is present to one specific condition of short duration and is only active in decomposing of organic matter present (Szekely *et al.*, 2009). All bacterial isolates were gram positive rods of different cell sizes (Table 1).

Microbiota of spent mushroom compost was investigated revealing the dominance of gram-positive bacterial isolates (Ntougias *et al.*, 2004). Majority of the bacterial isolates exhibited amylase, cellulase, and xylanase activity by producing clear halo zone on the plate. Isolates from airing stage were most active in cellulase, amylase and protease production (Table 2). All the isolates from casing soil produced siderophore and protease. However, the significant variation in xylanase secreting diversity was observed among the isolates. These observations indicate that every isolate and every stage of composting has different characteristics proving dynamic changes in composting process. Song *et al*. (2001) isolated thermophilic actinomycetes and Thermoactinomyces spp. from different mushroom compost, and performed their phylogenetic analysis. Microbial changes during the substrate preparation of *Agaricus bisporus* were monitored with molecular finger printing methods (Szekely *et al.*, 2009). They revealed the dominance of a supposedly cellulose-degrading consortium of mature compost composed of phylotypes related to Pseudo xanthomonas, Thermobifida, and Thermomonospora. The detailed profile may be observed in Table 2. Similar kind of study reported by Choudhary *et al*. (2009) who discussed the functional activity of casing amendments used in cultivation of *Agaricus bisporus*. These finding are in agreement with Singh *et al*. (2012) who described phylogenetic profiling of culturable bacteria associated with early phase of mushroom composting assessed by amplified rDNA restriction analysis

Antibiosis assay

In recent years, the researchers have focused on various microorganisms especially fluorescent *Pseudomonas* for biocontrol of mushroom diseases has been initiated. In this contest the antagonistic property of bacterial isolates against V*erticillium fungicola* and *Mycogone perniciosa* were checked in a dual culture assay. The fungus *Mycogone perniciosa* is reported to cause wet bubble disease in *Agaricus bisporus* in all the major growing countries of the world (Umar *et al.*, 2000), however V*erticillium fungicola* is a causal agent of dry bubble disease of mushroom and more severe than *Mycogone perniciosa* (Largeteau and Savoie, 2008). Results of antibiosis assay showed that all the isolates were neutral against *Mycogone perniciosa*. Twelve isolates were showing antagonism against *Verticillium fungicola*, however the intensity varied with the isolates (Table 2). Similar study with mesophilic bacterial population of early phase of mushroom composting was also described by Singh *et al*. (2012).

Solid state fermentation

White button mushroom (*Agaricus bisporus*) obtains nutrition from a selective, well –decomposed substrate prepared by consortium of microorganisms. Besides controlled degradation of specific metabolites are generated through microbial growth that determines the final quality of the compost (Johri and Rajni, 1999). In this contest, nine potential bacterial isolates were selected for solid state fermentation on the basis of qualitative enzymes assays and the mixture of the isolates were inoculated on '0' day mushroom compost for the quantitative enzyme assay of cellulase, xylanase, amylase and protease. During assay maximum activity was recorded for the amylase enzyme, which was increased by 1.9 and 2.1 fold over control,

Table.1 Morphological characteristic of bacterial isolates

*Bacterial isolate	Gram reaction	Cell shape	Cell size (µm)	Arrangement	Endospore formation	Colony Shape	Edge	Elevation	Surface	Chromogenicity
EA	+ve	Long rod	4.3	Chain	-	Irregular	Irregular	Flat	Rough	Creamish
BA	+ve	Thin rods	3.2	Chain	+	Irregular	Irregular	Flat	Glistening	Creamish
DA	+ve	Thin rods	3.12	Isolated	+	Irregular	Irregular	Flat	Glistening	Creamish
HA	+ve	Short rods	2.5	Chain	+	Circular	Entire	Flat	Glistening	Creamish
GD	+ve	Short rod	2.6	Chain	+	Irregular	Undulate	Flat	Glistening	White
ED	+ve	Rod	3.3	Isolated	-	Circular	Entire	Convex	Glistening	Red
FD	+ve	Thin rod	3.8	Isolated	+	Circular	Undulate	Flat	Glistening	Creamish
DD	+ve	Thin rod	3.5	Chain	-	Circular	Entire	Flat	Glistening	Creamish
DSR	+ve	Thin rod	3.4	Chain	-	Circular	Undulate	Convex	Smooth	Creamish
ASR	+ve	Thin rods	3.6	Chain	+	Irregular	Irregular	Flat	Glistening	Creamish
CSR	+ve	Thin rods	3.4	Diplobacillus	+	Irregular	Lobate	Flat	Glistening	Creamish
BSR	+ve	Thin rods	3.7	Chain	+	Irregular	Irregular	Umbonate	Glistening	Creamish
EC	+ve	Thin rods	3.8	Chain	-	Circular	Undulate	Flat	Glistening	Creamish
FC	+ve	Short rods	2.8	Chain	-	Circular	Entire	Flat	Glistening	Creamish
HC	+ve	Long rods	4.4	Isolated	-	Circular	Undulate	Umbonate	Smooth	Creamish
HCA	+ve	Short rods	2.4	Isolated	-	Circular	Undulate	Flat	Smooth	White
ICA	+ve	Rods	3.2	Isolated	-	Circular	Irregular	Flat	Rough	White
JCA	+ve	Rods	3.1	Isolated	-	Circular	Undulate	Flat	Rough	White
KCA	+ve	Rods	2.9	Isolated	-	Circular	Undulate	Flat	Rough	White
LCA	+ve	Long Rods	4.1	Isolated	+	Circular	Undulate	Flat	Rough	White

*Bacterial isolates: EA, BA, DA, HA isolated from Airing; GD, ED, FD, DD from Drenching; DSR, ASR, CSR, BSR from spawn run; EC, FC, HC from cropping stages of late phase of mushroom composting; and HCA, ICA, JCA, KCA, LCA were isolated from casing soil.

Table 2 Functional characteristic and antagonistic property of bacterial isolates

*Bacterial isolate	Functional characteristic					Antibiosis assay	
	Amylase	Cellulase	Xylanase	Protease	Siderophore	*Verticillium fungicola*	*Mycogone perniciosa*
EA	+	+	-	+	-	+++	-
BA	+	+	-	+	+	++	-
DA	+	+	-	+	+	++	-
HA	+	+	+	+	+	-	-
GD	-	+	+	+	+	-	-
ED	-	-	-	-	+	-	-
FD	-	+	+	+	+	-	-
DD	-	+	+	+	+	-	-
DSR	-	-	-	-	+	-	-
ASR	+	+	+	+	-	+	-
CSR	-	-	-	-	+	-	-
BSR	+	+	+	+	-	+	-
EC	-	-	-	-	+	+	-
FC	-	-	-	-	-	+	-
HC	-	-	-	-	+	++	-
HCA	+	+	+	+	-	+	-
ICA	+	-	-	+	+	++	-
JCA	+	-	-	+	+	++	-
KCA	-	-	+	+	+	-	-
LCA	-	-	+	+	+	+	-

-, No activity; +, Lower activity; ++, Moderate activity; +++, Maximum activity.

*Bacterial isolates: EA, BA, DA, HA isolated from Airing; GD, ED, FD, DD from Drenching; DSR, ASR, CSR, BSR from spawn run; EC, FC, HC from cropping stages of late phase of mushroom composting; and HCA, ICA, JCA, KCA, LCA were isolated from casing soil.

Table. 3 Enzyme activity after mixed culture inoculation under solid state fermentation

Enzymes	Un-autoclaved (UA)'0' day compost				Autoclaved (A) '0' day compost			
	Control		Inoculated		Control		Inoculated	
	Mean value*	Standard Deviation	Mean value*	Standard Deviation	Mean value*	Standard Deviation	Mean value*	Standard Deviation
Cellulase (IU/ml)	0.15	±0.005	0.23 (1.5)	±0.026	0.11	±0.012	0.2 (1.8)	±0.005
Xylanase (IU/ml)	0.45	±0.053	0.48 (0.0)	±0.02	0.08	±0.009	0.15 (1.87)	±0.009
Amylase (IU/ml)	0.93	±0.173	1.78 (1.9)	±0.187	0.66	±0.054	1.4 (2.1)	±0.282
Protease (IU/ml)	0.24	±0.009	0.29 (1.2)	±0.025	0.15	±0.007	0.25 (1.67)	±0.034
Reducing Sugars (µg/ml)	0.26	±0.008	0.3 (1.15)	0.018	0.09	±0.004	0.13 (1.45)	±0.013

* Mean value of three replicates; Values in parentheses indicate fold increase over control.

respectively under un-autoclaved (UA) and autoclaved (A) conditions (Table 3 and Figure.1). The cellulase, xylanase and protease activity was also increased over control (Figure. 1). The enhancement of enzyme activity under solid state fermentation mainly reported on the autoclaved '0' day compost and increased by 1.87, 1.8 and 1.67 fold over control, respectively for xylanase, cellulase and protease activity. However the maximum enhancement of cellulase and protease by 1.5 and 1.2 fold was measured under un-autoclaved condition (Table 3 and Figure. 1). Chang and Hudson (1967) reported solid state fermentation is a process of mushroom compost preparation which brought about the succession of microorganisms. These findings are agreement with Singh *et al*., (2012) who reported enhancement of enzyme activity under solid state fermentation on the autoclaved mushroom compost for xylanase, amylase and protease.

ARDRA analysis

Considering the significance of mushroom cultivation in the country and yet limited information on mushroom composting bacterial flora and their phylogenecity, an exhibitive analysis of mesophilic bacterial diversity of this unique material was undertaken. Microbial dynamics of composting in general has been well studied with various techniques (Klamer and Baath, 1998; Peters *et al.*, 2000; Takaku, 2006), but composting procedures are rather different from the faster and better regulated mushroom compost preparation. T-RFLP was also used in another report to study the bacterial community succession in compost preparation for Oyster mushroom (Vajna *et al.*, 2010). In present study, we used amplified ribosomal DNA restriction analysis (ARDRA) for the identification of mushroom compost bacteria. In this technique, 16S rDNA gene analysis was intensively performed to understand the phylogenetic relationship amongst all bacterial isolates. This molecular technique has been successfully used for community analysis in a great variety of environments (Lagace *et al.*, 2004; Li *et al.*, 2008; Abbate *et al.*, 2009; Song *et al.*, 2011).

Cluster analysis of 16S rDNA restriction profile

In this study twenty bacterial isolates, representing different stages of late phase of mushroom composting process were subjected to ARDRA by digestion of the amplified 16S rRNA gene with *Msp1, Sau 3AI* and *Taq1*. Three separate dendrogram of band pattern were obtained after three independent digestions. All the restriction profile based on dendrogram had shown specific similarity value ranged widely between 50- 100% (Figure.2).

Msp I digestion of amplified 16S rDNA classified these twenty bacterial isolates into 13 groups; of these five groups were 100% similar. First group included 2 isolates of casing soil (JCA and KCA). Second group included 4 isolates of which the two (DSR and BSR) were from spawn run and the other two (DA belonged to airing and FD to drenching stage) belonged to different stages. Third group had two isolates (HA and DD belonging to airing and drenching stages respectively). Fourth group also included two isolates (EA and HCA belonging to airing and casing soil respectively). Fifth group had two isolates (FC and EC) belonging to cropping stage of mushroom compost (Figure . 2A).

Figure.1 Enzyme activity of mixed culture under solid state fermentation.

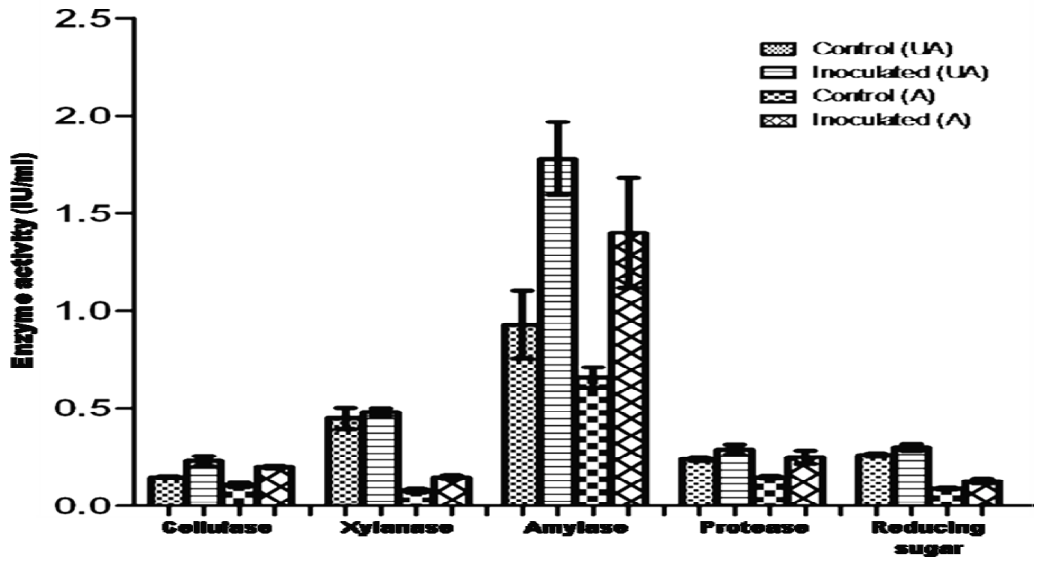
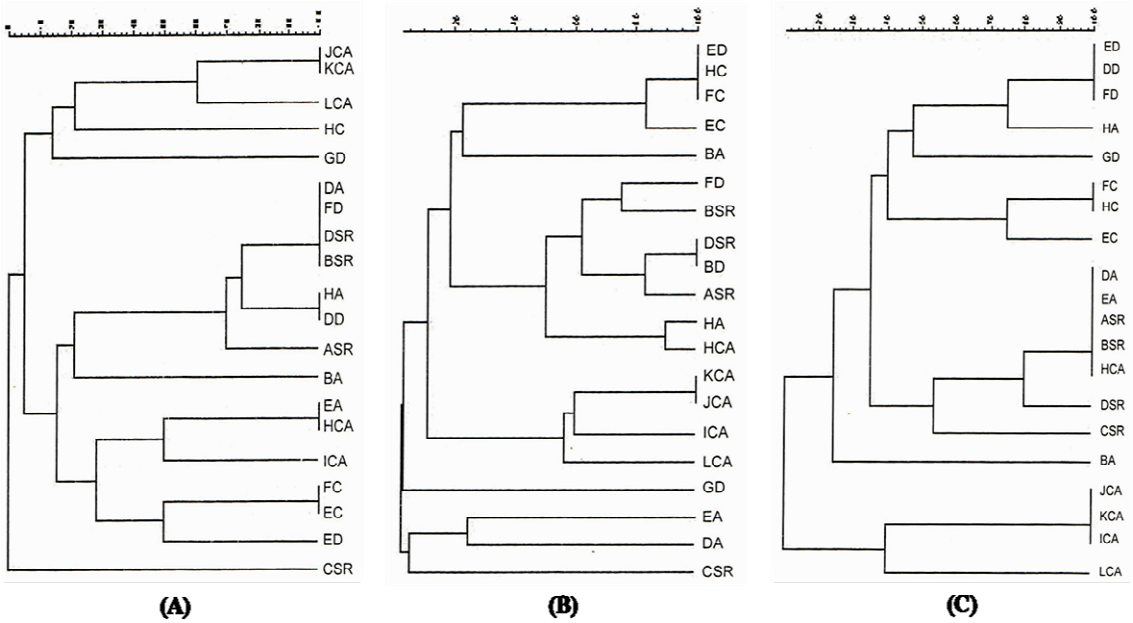


Figure.2 Comparative UPGMA dendrogram analysis of bacterial isolates based on amplified 16S rDNA restriction profile: **(A)** - Digested with *Msp 1*, **(B)** - *Sau 3AI*, **(C)** - *Taq 1*.



Consortium of selected bacterial isolates produced good amounts of lytic enzymes (amylase, cellulase, xylanase and protease) in solid state fermentation experiment that might help in enhancing composting process. 'UA' and 'A' stands for unautoclaved and autoclaved respectively.

Sau 3AI digestion classified these isolates into three groups which were 100% similar. First group included three isolates (HC and FC belonged to cropping and ED to drenching stage). Second group had two isolates (DSR and BD from spawn run and drenching stages respectively). Third group also included two isolates (KCA and JCA from casing soil) (Figure . 2B).

Taq I digestion classified into four groups which were 100% similar. First group included three (FD, ED and DD) belonging to drenching and second group had two isolates (FC and HC) from cropping stage. Third group included five isolates (DA and EA belonged to airing, ASR and BSR from spawn run and HCA belonged to casing soil). Fourth group included three isolates (JCA, KCA and ICA) and all belonged to casing soil (Figure . 2C).

Comparative analysis of UPGMA dendrogram of Msp I, Sau 3AI and Taq I

Most of the isolates were clearly differentiated by these three enzymes used in this study. Isolates JCA and KCA were 100% similar on analysis by all three enzymes. On the other hand, FC and HC showed 100% similarity when they were restricted by Taq I and Sau 3AI. All other isolates were observed to be different (Fig. 2). These finding clearly indicate that there is a diverse bacterial population which is involved in composting process and one population is particularly involved in one stage and replaced by other bacterial population showing a very good example of community succession (Vajna *et al.*, 2010). These results are also supported with the finding of Singh *et al*. (2012) who described phylogenetic profiling of culturable bacteria for early phase of mushroom composting.

This is the first preliminary report on the morphological, functional and genetic characterization of mesophilic bacteria for late phase of mushroom composting presenting a very good example of community succession. We further found that microbial inoculants can improve the conditions of compost for growth of mushroom by enhancing the rate of degradation of complex organic residue, process of solid state fermentation, spawn run in the substrate and in disease management.

Acknowledgements

Financial support from Ministry of Environment and Forest, Government of India, New Delhi to the Centre for Research on Bacteria and Archaea (CRBA) is gratefully acknowledged. Authors thank to Joint Director Dr. R. P. Singh, Mushroom Research and Training Centre, G.B. Pant University of Agriculture and Technology, Pantnagar for supply of compost.

References

Abbate, C., M. Arena, A. Baglieri and Gennari, M. 2009. Effects of organoclays on soil eubacterial community assessed by molecular approaches. J. Hazard. Mater. 168: 466-472.

Bazzicalupo, M., and Fani, R. 1994. The use of RAPD for generating specific DNA probes for microorganisms. In: Clapp, J. (ed) Methods in molecular biology.

Humana Press Inc, Totowa, New Jersey, USA, pp. 155-175.

Beffa, T., M. Blanc and Arango, M. 1996. Obligately and facultatively autotrophic, sulfur and hydrogen oxidizing thermophilic bacteria isolated from hot compost. Arch. Microbiol. 62: 1723-1727.

Blanc, M., L. Marilley, T. Beffa and Aragns, M. 1997. Rapid identification of heterotrophic thermophilic, spore forming bacteria isolated from hot compost. Int. J. Bacteriol. 47: 1246-1248.

Chadha, B.S., S. Singh, G. Bohra and Saini, H.S. 1997. Shake culture studies for the production of amylase by *Thermomyces lanuginosus*. Acta. Microbiol. Immunol. Hung. 44: 181-185.

Chang, Y., and Hudson, H. J. 1967. The fungi of wheat straw compost: Ecological studies. Trans. Brit. Mycol. Soc. 50: 649-666.

Choudhary, D. K., P. K. Agarwal and Johri, B. N. 2009. Characterization of functional activity in composted casing amendments used in cultivation in *Agaricus bisporus* (Lange) Imbach. Indian J. Biotechnol. 8: 97-109.

Fermor, T.R.., and MaCauley, B. J. 1991. Microbiological factors contributing to selectivity and nutritional quality of mushroom compost. *In:* Nair, N.G. (ed). Proceedings of AMGA/ISMS. International Workshop – Seminar on Agaricus compost AMGA, Sydney, pp. 48-67.

Holt, J. G., N. R. Krieg, P. H. A. Sneath, J. T. Staley and Williams, S. T. (1994) Bergeys manual of determinative bacteriology, 9th Edn. Williams and Wilkins, USA.

Johri, B.N., and Rajni. 1999. Mushroom compost: microbiology and application. *In:* Bagyaraj, D.J.; Verma, A.; Khanna, K. K. (eds). Modern approaches and innovation in soil management. Rastogi Publication, Meerut, India, pp. 345-358.

Klamer, M., and Bååth, E. 1998. Microbial community dynamics during composting of straw material studied using phospholipid fatty acid analysis. FEMS. Microbiol. Ecol. 27: 9–20.

Lagace, L., M, Pitre, M, Jacques and Roy, D. 2004. Identification of the bacterial community of maple sap by using amplified ribosomal DNA (rDNA) restriction analysis and rDNA sequencing. Appl. Environ. Microbiol. 70: 2052-2060.

Largeteau, M. L., and Savoie, J. M. 2008. Effect of the fungal pathogen *Verticillium fungicola* on fruiting initiation of its host, *Agaricus bisporus*. Mycol. Res. 112: 825-828.

Li, Z., C. Zou, Y. He, M. Mo and Zhang, K. 2008. Phylogenetic analysis on the bacteria producing non-volatile fungistatic substances. J. Microbiol. 46: 250-256.

Lowry, O. H., N. J. Rosebrough, A. L. Farr and Randall, R. J. 1951. Protein measurements with the folin phenol reagent. J. Biol. Chem. 193: 265-275.

Masaphy, S., D. Levanon, O. Danai and Henis, Y. 1987. Nutritional supplementation to the casing soil: Ecological aspects and mushroom production, Mushroom Sci Part II. XII Int Congress on Science and Cultivation of edible fungi, Braunschweig, Germany, pp. 417-426.

Miller, G. L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31: 426-428.

Ntougias, S., G. I. Zervakism, N. Kavroulakis, C. Ehaliotis and Papadopoulou, K.K. 2004. Bacterial diversity in spent mushroom compost assessed by amplified rDNA restriction analysis and sequencing of cultivated isolates. Syst. Appl. Microbiol. 27: 746–754.

Peters, S., S. Koschinsky, F. Schwieger and Tebbe, C. 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: 930–936.

Schwyn, B., and Neilands, J. B. 1987. Universal chemical assay for the detection and determination of siderophores. Anal. Biochem. 160: 47-56.

Silva, C. F., R. S. Azevedo, C. Braga, R. Silva, E. S. Dias, Schwan, R. F. 2009. Microbial diversity in a bagasse-based compost prepared for the production of *Agaricus brasiliensis*. Brazilian. J. Microbiol. 40: 590-600.

Singh, A. V., A. Sharma and Johri, B. N. 2012. Phylogenetic profiling of culturable bacteria associated with early phase of mushroom composting assessed by amplified rDNA restriction analysis. Ann. Microbiol. 62: 675–682.

Song, J., H. Y. Weon, S. H. Yoon, D. Park, S. S. J. Go, Suh, J. W. 2001. Phylogenetic diversity of thermophilic actinomycetes and *Thermoactinomyces* spp. Isolated from mushroom composts in Korea based on 16S rRNA gene sequence analysis. FEMS. Microbiol. Lett. 202: 97–102.

Song, Y., Z. Liu, J. Han, Hu, Y. 2011. Culturable bacterial community analysis in the root domains of two different tree peony (*Paeonia ostii*) variety plants. FEMS. Microbiol. Lett. 10: 1574-6968.

Straatsma, G., R. A. Samson, T. W. Olijnsma, H. J. M. Oplen Camp, J. P. G. Gerrits, Van Griensven L. J. L. D. 1994. Ecology of thermophilic fungi in mushroom compost, with emphasis on *Scytalidium thermophilum* and growth stimulation of *A. bisporus* mycelium. Appl. Environ. Microbiol. 60: 454-458.

Szekely, A. J., R. Sipos, B. Berta, B. Vajna, C. Hajdu and Marialiget, K. 2009. DGGE and RFLP analysis of bacterial succession during mushroom compost production and T-RFLP profile of mature compost. Microb. Ecol. 57: 522-533.

Takaku, H., S. Kodaira, A. Kimoto, M. Nashimoto and Takagi, M. 2006. Microbial communities in the garbage composting with rice hull as an amendment revealed by culture-dependent and independent approaches. J. Biosci. Bioeng. 101: 42–50.

Umar, M. H., F.P. Geels and Van Griensven, L. J. L. D. 2000. Pathology and pathogenesis of *Mycogone perniciosa* infection of *Agaricus bisporus*, *In:* XV International Congress on the Science and Cultivation of Edible Fungi, Maastricht, Netherlands, 15-19 May, 2: 561-567.

Upton, M. E., and Fogarty, W. M. 1977. Production and purification of theromstable amylase and protease of *Thermomonospora viridae*. Appl. Environ. Microbiol. 33: 59-64.

Vajna, B., A. Nagy, E. Sajben, L. Manczinger, N. Szijártó, Z. Kádár, D. Bordás and Márialigeti, K. 2010. Microbial community structure changes during oyster mushroom substrate preparation. Appl. Microbiol. Biotechnol. 86: 367–375.

Weisburg, W. G., S. M. Barns, D. A. Pelletier and D. J. Lane. 1991. 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol. 173: 697-703.

Wood, D. A., and Fermor, T. R. 1981. Nutrition of *Agaricus bisporus* in compost. Mushr. Sci. 11: 63.