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ORIGINAL ARTICLE

Evaluation of *in situ* functional activity of casing soils during growth cycle of mushroom (*Agaricus bisporus* (Lange) Imbach) employing community level physiological profiles (CLPPs)

Devendra Kumar Choudhary · Pavan K. Agarwal · Bhavdish N. Johri

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Abstract Community level physiological profiles (CLPPs) have been rarely applied to mushroom compost ecosystem, probably for the lack of standardized methodology. Recently, however CLPPs have been employed as a tool to investigate the degree of maturity of compost (Mondini and Insam, 2005, Compost Science and Utilization, 13(1): 27–33). The potential of CLPPs to detect compost maturity test is considerably significant in that it provides sensitivity and the simplicity of the assay. The aim of this work was to investigate the maturity of casing that comprised of farm yard manure and spent compost and influence of casing type on the behaviour of bacterial community during the growth cycle of mushroom *Agaricus bisporus* (Lange) Imbach employing standardized inoculum density and effects of different data interpretation based on the kinetics of colour formation. Casing samples of different age were extracted at a particular dilution and then inoculated in 96 well microtitre plates. Optical density (OD) in well was measured at 590 nm every 24 hours for 5 days. Principal component analysis (PCA) was performed by employing OD values at fixed average well colour development (AWCD). PCA of fresh samples showed that classification and ordination of samples according to their age were significant with fixed AWCD.

Keywords Community-level physiological profile · Mushroom cropping · PCA · AWCD · Diversity indices · Community succession

D. K. Choudhary[1] (✉) · P. K. Agarwal[2] · B. N. Johri[3]
Department of Microbiology,
GB Pant University of Agriculture & Technology,
Pantnagar - 263 145, Uttarakhand, India
[1]Centre for Scientific Research & Development (CSRD),
People's Group, By-pass Road,
Bhanpur, Bhopal - 462 010,
India
[2]Department of Biotechnology,
Sai PG Institute of Paramedical & Allied Sciences,
Dehradun, Uttarakhand, India
[3]Department of Biotechnology & Bioinformatics Centre,
Barkatullah University,
Bhopal - 462 026, MP, India

E-mail: devmicro@rediffmail.com

Introduction

The mushroom *Agaricus bisporus* (Lange) Imbach has a requirement for "casing layer" that stimulates and promotes the initiation of primordia thereby enhancing yield of by increasing the number of flushes during growth cycle; this is on account of unique physico-chemical and microbiological properties of the casing layer [1]. Mushroom cropping constitutes four successive stages that include zero day casing (when casing was applied over compost with fully colonized mycelium), mycelium-impregnated stage (colonization of casing by mycelium), primordial stage (when primordia protrude over casing), and harvesting stage (when flushes (sporophores) are picked up). Microorganisms in casing play a crucial role in initiation and development of primordia and more uniform distribution of sporophores, higher yield of mushroom and early completion of cropping. There is a succession of different microbial populations in successive stages of mushroom cropping influenced by casing type that affect yield of mushroom.

Microbial communities have great potential for temporal and spatial change, and thus represent a powerful tool

for understanding community dynamics in both basic and applied ecological contexts. The small size and rapid growth of microorganisms allow for complex community interactions to be studied much more readily than with plants or animals. Variation in microbial community structure may influence ecosystem processes (e.g. nutrient recycling, decomposition) or the effectiveness of microbial invasions (e.g. growth of pathogens, release of genetically engineered microorganisms). Understanding how community processes affect ecosystem processes poses a central challenge in ecology, and microbial communities offer a potentially powerful forum for advancing this understanding [2].

The potential of CLPPs to check the functional activity in mushroom cropping is significant in that the assay is sensitive and simple. Mondini and Insam [3] applied CLPPs approach to detect compost maturity. Nevertheless, CLPPs of composting processes have been studied only in limited cases and it requires special care to obtain realistic and reproducible results because of fast changes in physico-chemical and microbiological properties, the high spatial heterogeneity and the colouration of the extracts [4, 5, 6].

Differences in inoculum density alter the rate of colour formation and comparison of casing samples employing multivariate analysis at a single incubation time may yield a classification of samples based on the overall amount rather than the relative pattern of carbon source utilization. To eliminate the confounding effects of the inoculum density, Garland [7] has proposed an approach that is based on appropriate standardization of inoculum density, repeated reading of the plates and subsequent selection of readings closest to a given AWCD for analysis.

Since, the colour development typically follows a sigmoidal trend with time, therefore CLPPs interpretation based on the kinetics of colour formation rather than on the degree of colour development at a given time. CLPPs suggested giving a more effective description of the whole-community substrate utilization patterns [2]. In vitro community level physiological profiles (CLPPs) are based on the capacity of microorganisms to utilize different carbonaceous substrates and have been widely successfully used to characterize microbial communities from different habitats [8, 9].

The CLPP can be analyzed by generating variables such as colour intensity (absorbance) or binary data based on presence or absence of colour development in each microplate well. Distinctive colour patterns in the reaction well array have been reported and described for soils from different ecosystems and plant communities as well as agricultural soil with different management regimes [10, 11, 12]. As an example, Goodfriend [13] determined that microbial community substrate utilization reflected similarity in habitat type rather than geographical influences in different saline systems (salt marsh, sand dune, and seawater irrigated agronomic systems). Limited studies have been made on soil microbial community activity due to consistent long-term experimental soil management within a site or to the flexible on-farm soil management and no work has been done on casing soil.

Therefore, the aim of this work was to investigate the suitability of CLPPs data interpretation based on the kinetics of colour formation as applied to successive stages of mushroom cropping samples to evaluate whole bacterial community that may be influenced by casing type.

Materials and methods

Sample and sampling site

Samples (farm yard manure and spent compost individually and in the ratio of 3:1 and 2:1 respectively) were collected by removing casing layer aseptically into polyethylene bags from two stages (0 day and harvesting stage) of mushroom cropping from the Mushroom Research and Training Centre (MRTC) of the University (GB Pant University of Agri. and Tech., Pantnagar, India).

Sample preparation

Two different combinations of two type of casing (FYM and SC) layer from 0 day stage and mushroom final harvesting stage were selected for community level physiological profile. These included, FYM + SC (3:1) and FYM + SC (2:1), FYM alone and SC alone. CLPP analysis was performed to check the differences in patterns of potential carbon source utilization during the microbial succession. Multiple composite of casing sample (10 g) was suspended in 90 ml of 0.85% saline with several glass beads in 200 ml volumetric flask and placed on a reciprocating shaker at 120 rpm for 60 min; appropriate dilution (10^{-4}) [14] was added to 96-well microplates.

Microplates inoculation and plate reading

Carbon source utilization trials used microtiter plates. Plate wells were filled with 100 µl of C-source (0.1 mg), 50 µl of triphenyl tetrazolium chloride (TTC) (250 µg) and 50 µl of minimal medium (M9) (NH_4Cl 0.1%, NaCl 0.05%, KH_2PO_4 0.3%, Na_2HPO_4 0.6%, 1M $MgSO_4.7H_2O$ (2 ml/l) and 1M $CaCl_2$ (0.1ml/l), devoid of C-source. Each well was inoculated with 50 µl of a suspension of casing samples in a final dilution of 10^{-4}.

Table 1 List of variables used for community-level physiological profiling

Carbohydrates and Derivatives	Amino Acids and Derivatives
Inulin	L-Methionine
Cellobiose	L-Arginine
L-Arabinose	L-Glutamic acid
Laminarin	Glycine
D-Arabinose	L-Tyrosine
D-Galactose	Leucine
D-Xylose	L-Cysteine hydrochloride
Glucose	L+Arginine
D-Mannitol	L-Tryptophane
Soluble Starch	Asparagine monohydrate
Rhamnose	L-Lysine
Maltose	Phenylalanine
Trehalose	DL-Serine
Mannane	DL-Valine
D(+)-Mannose	L-Aspartic acid
	L-Valine

The microtiter plates contained three replicate wells of 31 carbon substrates in random manner; these substrates were amino acids (n = 16) and carbohydrates (n = 15) that were ecologically relevant to mushroom compost (Table 1). A control in three replicate well contained no sole-carbon substrate. Plates were incubated at 20 °C in the dark. Colour formation in microplate wells in CLPP is based on the conversion of the redox-sensitive tetrazolium dye which is reduced during respiratory activity, and accumulates as insoluble formazan inside active cells. The optical density at λ = 590 nm was determined because the peak absorbance of tetrazolium dye occurs at 590 nm. An incubation time of 120 h was used in all cases to allow growth of slower-growing microbes or of those present in low numbers in the inoculant [15]. Colour development was measured at 590 nm with a model Spectra Max Microplate reader no. 340.

Analysis of microplate data

Two analytical approaches were used to analyse the microplates data. One approach was to use a measure of total activity or bioactivity for microtitre plates such as AWCD and other approach was used to quantify substrate diversity as Gomez et al. [16]:

$$H = -\Sigma Pi (\ln Pi)$$

where Pi is the ratio of activity on a particular carbon source to the sum of activities on all substrates. Average well colour development (AWCD) was calculated according to Garland and Mills [8], i.e.,

$$AWCD = \Sigma (C–R)/n$$

where C is colour production within each well (optical density measurement), R is the absorbance value of the plate's control well, and n is the number of substrates (Microplates, n = 31). Mean AWCD values were plotted overtime for each sample. Negative values were considered as 0 in subsequent data analyses [17].

For principle component analysis (PCA), data were first transformed by dividing, for each substrate, the difference in optical density at 120 h, relative to control well, by the AWCD of the plate at 120 h, i.e. (C – R / AWCD) [17]. Principle component analysis (PCA) was performed on the transformed AWCD. Multivariate statistical techniques were necessary to compare samples given the large number of variables (31) per samples. PCA was conducted using software S-Plus ver. 6.0 Insightful Inc., USA.

The purpose of ordination was to arrange samples of multidimensional space into a low-dimensional space such that similar samples were closeby and dissimilar samples were far apart [18]. PCA was employed by Garland and Mills [8, 19], and is commonly used to reduce complex multidimensional data into smaller number of interpretable variables, or principle components, that represent a subset of the original variables. PCA projects original data onto new, statistically independent axes (PC). Each principle component (PC) extracts a portion of the variance from the original data.

Results and discussion

Community level physiological profile

Assessment of community level bacterial functionality was performed employing sole carbon source utilization pattern in a 96 well ELISA plate containing minimal medium. Average colour development of the plates was read on an ELISA reader and computed to derive at the functionality of the in situ casing soil community.

Variation of AWCD

Colour development (expressed as AWCD) followed a sigmoidal curve with incubation time (Fig. 1). In all plates, AWCD values in the first 24 h showed little colour change, however with time colour development proceeded in patterns that were linear and non-linear. Samples from FYM casing and FYM + SC (2:1) at 0 day and harvesting stages

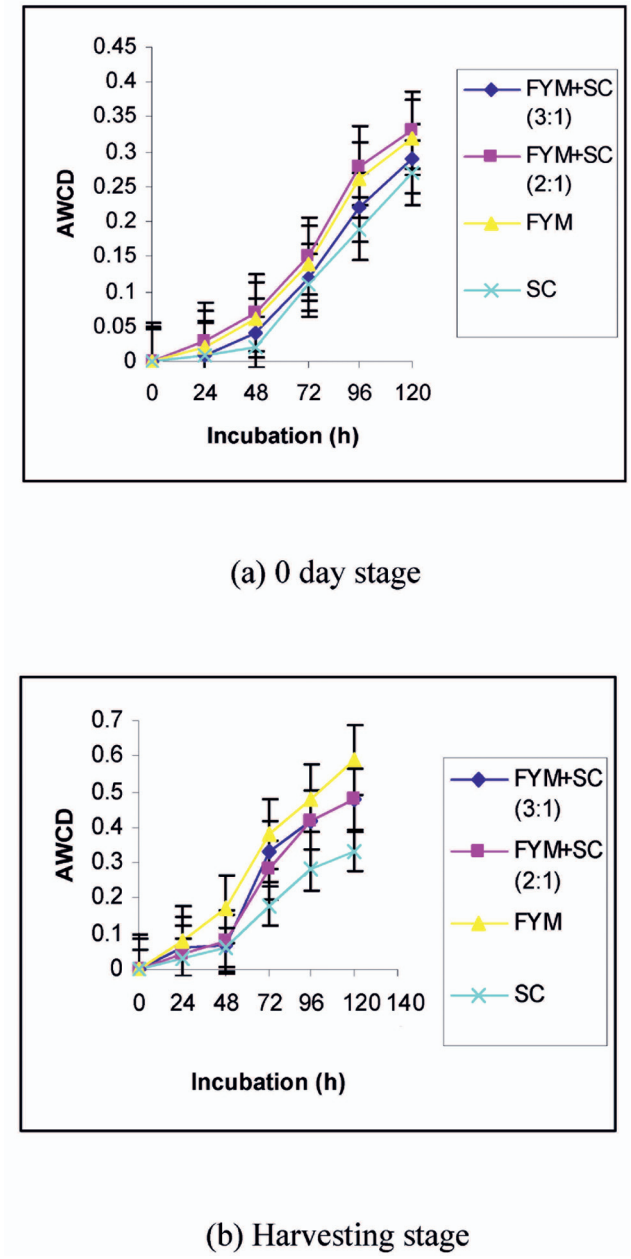

Fig. 1 Variation in average well color development (AWCD) over time for (a) 0 day stage and (b) harvesting stage casing soil samples.

showed highest colour development (AWCD). The AWCD values for FYM + SC (3:1) casing, and spent compost casing were intermediate for 0 day and harvesting stages

Functional biodiversity

Based on the AWCD data, species indices and compositional similarity for in situ diversity was calculated. Richness (R_i), as the number of oxidized C substrates, and the Shannon-Weaver index (H') (i.e., the richness and evenness of response) were calculated from the data obtained at 120 h incubation of plates. Similarly, substrate evenness (E) measures the equitability of activities across all utilized substrates and is given by:

$$H = H/H_{max} = H/\log S$$

Where H is substrate diversity and S, substrate richness. Differences in the number of utilized substrates within the 0 day and harvesting stage samples, demonstrated a temporal dimension of functional diversity that developed during incubation. Both, functional richness and total activity were high for FYM casing and FYM+ SC (2:1) casing samples of 0 day and harvesting stages. Samples from FYM + SC (3:1) casing and spent compost casing (0 day and harvesting stages) showed intermediate substrate diversity. Variation was found in evenness among samples**.** It remained uniform throughout the incubation period with little variation for the casing soil samples at 0 day and harvesting stages. The variation in evenness was found because of substrate richness, i.e., the number of C-substrates oxidized.

Compositional similarity

Measures of substrate richness, evenness and diversity do not provide information about the types of substrates that are utilized by the bacterial community. Two sites could exhibit identical substrate richness, evenness or diversity but still catabolize totally different substrates.

Cluster analysis, based on the presence or absence of utilized substrates revealed consistent relationship among the samples of 0 day and harvesting stages (Fig. 2). Samples

Table 2 Functional diversity in casing for 0 day and harvesting stage samples

Diversity index	0 day stage				Harvesting stage			
	FYM casing	Spent compost casing	FYM+SC (2:1) casing	FYM+SC (3:1) casing	FYM casing	SC casing	FYM+SC (2:1) casing	FYM+SC (3:1) casing
[a]H'	2.7	2.35	2.85	2.49	3.1	2.5	2.88	2.7
[b]E	1.98	2.08	2.22	2.0	2.06	2.02	1.94	2.2

a = Shannon-Weaver diversity index.
b = Substarte Evenness.

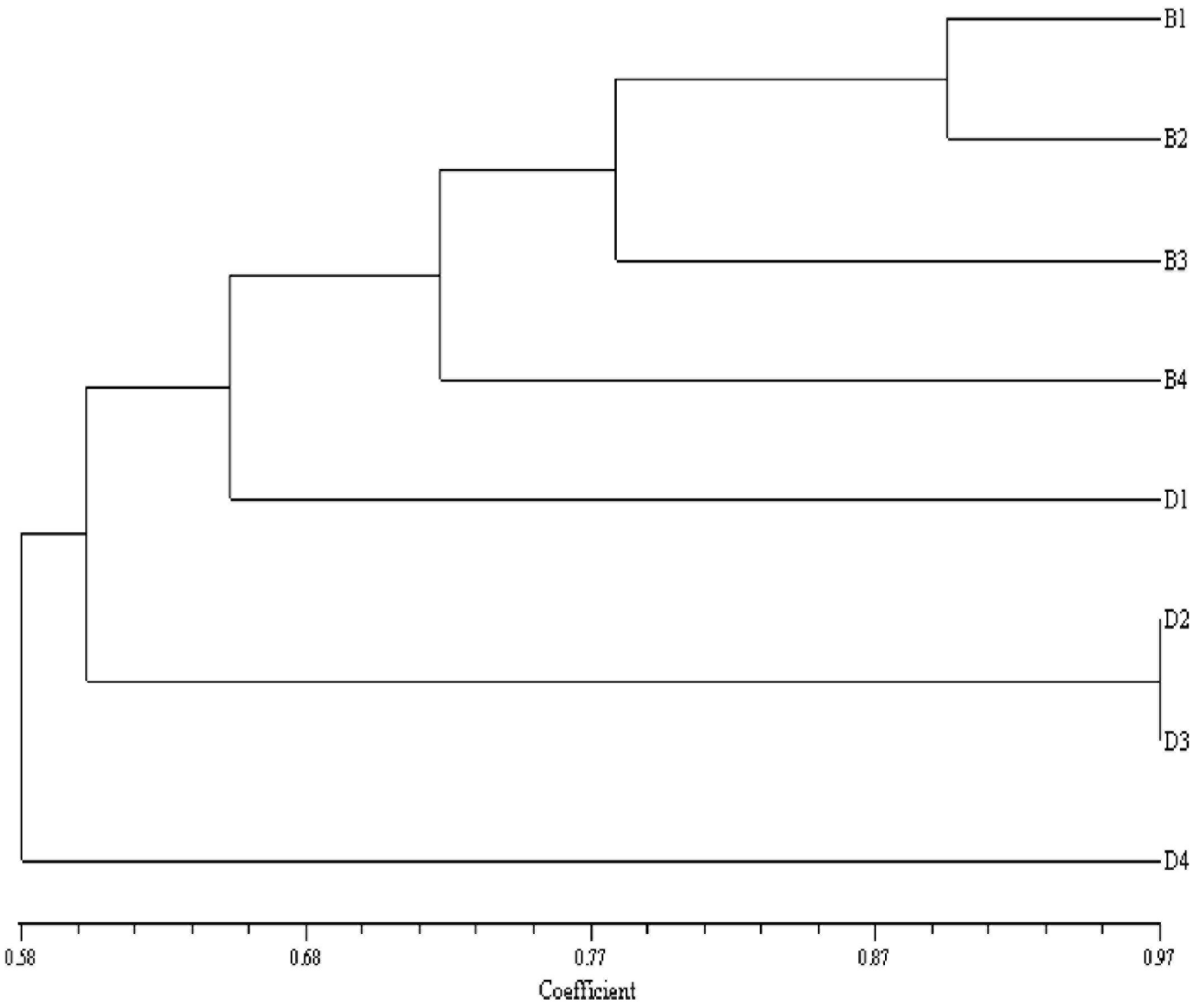

Fig. 2 Compositional relationship among casing soil samples based on overall substrate utilization using cluster analysis (presence or absence of activity). Letters and numerals represents samples recovered from different casing soils and their stages respectively, i.e., B1=FYM+SC (3:1); 0 day stage, B2= FYM+SC (2:1); 0 day stage, B3=FYM; 0 day stage; B4=SC; 0 day stage, D1= FYM+SC(3:1) ; harvesting stage , D2= FYM+SC(2:1); harvesting stage , D3= FYM; harvesting stage and D4=SC; harvesting stage. The dendrogram was based on Jaccard's similarity coefficient by employing UPGMA analysis.

of FYM + SC (3:1) and FYM + SC (2:1) casing soils recovered from 0 day stage were indistinguishable from each other i.e., they had 100% similarity. On the other hand, 90% similarity was observed for FYM + SC (2:1) and FYM casing soils from harvesting stage. The greatest dissimilarity was observed for SC samples; it showed only 58% similarity with other samples. Ordination (principal components analysis) of the 0 day and harvesting stage communities based on activity levels also demonstrated a consistent relationship within the communities throughout the incubation period. This was irrespective of the fact that the number of catabolized substrates and levels of activity in the 0 day and harvesting stage communities changed substantially during the incubation period. The PCA plot produced a pattern of inter-site relationships, similar to that of cluster analysis.

End-point AWCD approach

Principal component analysis (PCA) of transformed, 120 h AWCD data successfully distinguished within the casing soil samples of 0 day and harvesting stages that expressed heterotrophic bacterial activity in the given casing niche. Samples from casing soils (0 day and harvesting stage) had distinctive patterns of sole-C-source utilization on the basis of PCA of transformed colour response data.

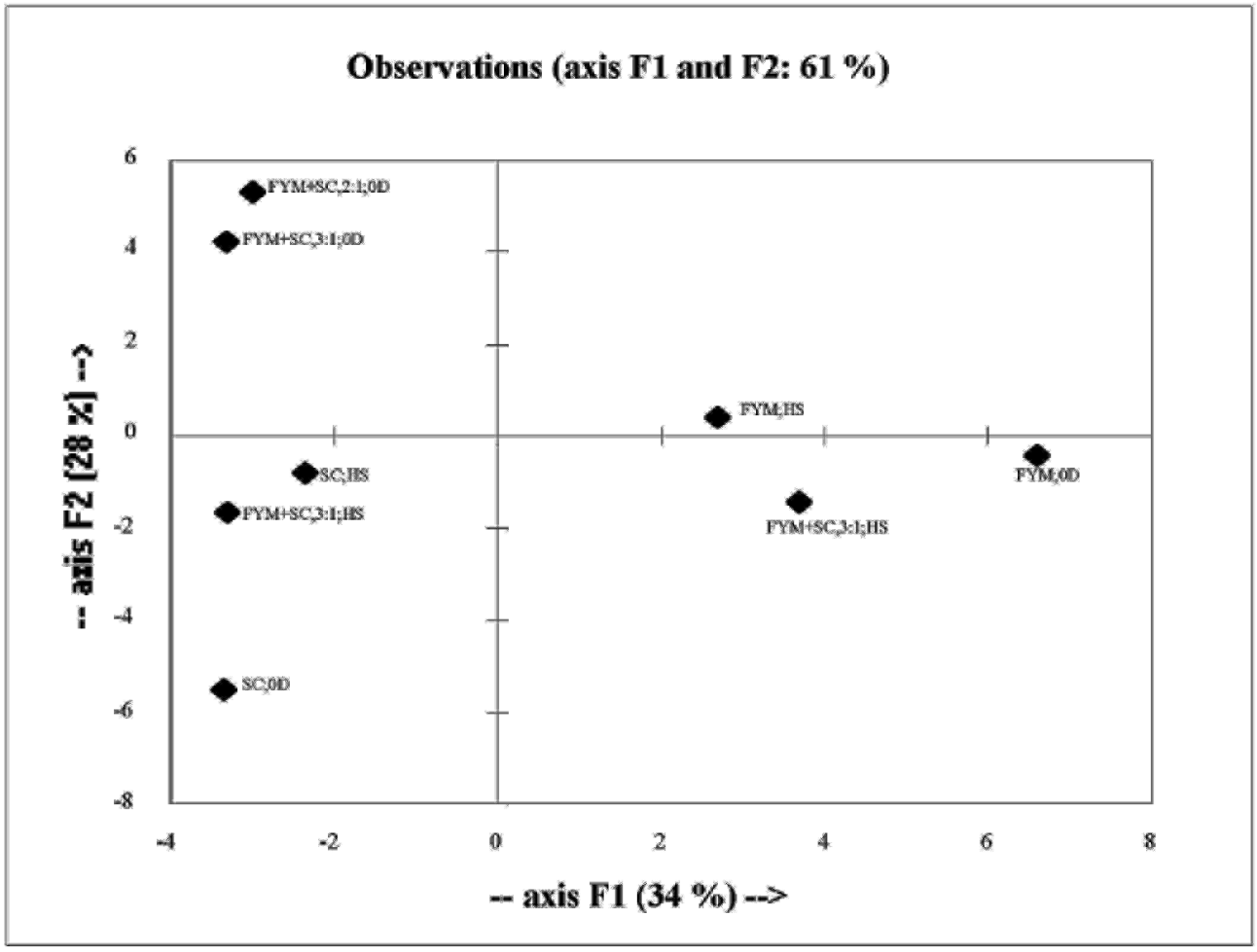

Fig. 3 Multivariate classification of casing soils sample based on carbon substrate utilization in microtiter plate. Analyses represent PCA of average well color development (AWCD) at 120 h. Where 0D encodes stage at the time of casing and HS encodes stage for the time of harvesting.

Differences among casing soil samples

Casing soil samples viz., SC of 0 day, FYM + SC (3:1) and SC of harvesting stages had much lower coordinate values (PC scores) for the first PC, which explained 34% variance in the data, compared to other casing soil samples from 0 day and harvesting stage. Samples from the later two stages had similar value for PC 1, but FYM + SC (3:1) and FYM + SC (2:1) had different PC scores along the second PC, with the FYM casing soil at harvesting stage possessing higher coordinate values. The second PC explained 28% of the variance in the data. Samples with higher coordinate values for PC 1 showed a greater response for C-sources, and correlated positively to PC 1 as compared to sample with low PC scores (Fig. 3).

Analysis of PC 1 indicates that SC (0 day casing), FYM + SC (3:1) casing and SC from harvesting stage utilized a number of carbohydrates (L-arabinose, laminarin, D-xylose, mannane, D-arabinose) and amino acids (DL-valine, phenylalanine, L-tyrosine, glycine and L-arginine) to a relatively lesser degree than other casing soil samples (0 day and harvesting stage). On the basis of PC 2 analysis, bacterial community associated with FYM + SC (3:1) and FYM + SC (2:1) showed relatively greater utilization of four carbohydrates (inulin, D-galactose, glucose, D-mannitol) and two amino acids (leucine and DL-serine). Similarly, FYM casing (0 day), FYM + SC (3:1) casing and FYM casing (harvesting stage) showed greater utilization of cellobiose, L-glutamic acid, L-cysteine hydrochloride, L-arginine, maltose, L-lysine, trehalose, D(+)-mannose, and L-valine on the basis of PC 1 analysis.

Recent advances in the estimation of prokaryotic diversity have brought into focus two particular questions: what is the extent of prokaryotic diversity and why bother finding

out this diversity? The global reservoirs of diversity are an important driving force behind patterns in localized diversity. Where the reservoir community is large and relatively even, chance alone will prevent physically identical communities from having the same, or sometimes even stable communities. Communities that tend to be similar and stable are observed where the source diversity is low. Thus, the relationship between structure and function in a community can only be understood, predicted and engineered through an understanding of the source of diversity from which the community is drawn [20].

Due to their physiological diversity, microorganisms play major roles in the cycling of chemical elements within the biosphere. Empirical investigations of interdependencies (impact of microbial diversity on ecosystem) depend unavoidably on the evaluation of biodiversity in quantitative terms. This quantification had not yet been achieved on the basis of the new molecular methodologies [21] for many ecosystems, but in principle it is possible and probably desirable [22] that such information be collected for posterity.

Considering the significance of mushroom production in the country and yet, rather ill-defined fingerprint of the all important casing soil, an exhibitive analysis of bacterial diversity of this unique material was undertaken. A comparison of the relative ability of a microtiter plate assay using multiple substrates was used to distinguish among heterotrophic bacterial communities from four different casing soils at 0 day and harvesting stages. When the transformed colour development data was projected onto two-dimensional principal component space, the percentage of overall variation explained by the first two components was higher for data from 0 day and harvesting stage casing soils i.e., 60%. To some degree, the same substrates loaded prominently on the first principal component (PC1) helped calculate the data but PC2 component showed marked differences among casing soil samples. The heterotrophic bacteria from 0 day stage casing soil samples exhibited more variable colour development, relative to bacteria from harvesting stages. Available data showed that the functional abilities of casing soil community changed during succession.

Pattern analysis of carbon-source utilization had been proposed as a simple and rapid method to characterize heterotrophic microbial communities [23, 24]. Multivariate analysis of the profiles has been used to distinguish among samples from different habitats [19], soil type [25, 14] and size fractions within the same soil [25]. The reference point was used to measure the colour development (AWCD), where it was 0.75 – 1.00. The specific reference point in AWCD does not appear to be critical for classification of samples. The C-sources responsible for the differences between samples varied with reference point [26]. As more colour developed in the well (AWCD), the functional diversity of bacterial community was enhanced i.e., AWCD had linear relationship with H¢. Thus, high values of the Shannon index in microtiter plates showed high functional diversity and low value showed lower functional diversity. A distinct relationship between AWCD and the two measures of diversity (the Shannon index and the Gini coefficient) indicated that to have high levels of total activity on a microtiter plate (AWCD), the majority of the C-sources would also have to be utilized and at similar rates (the Shannon index and the Gini coefficient) [27].

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References

1. Noble R, Fermor TR, Lincoln S, Dobrovin-Pennington A, Evered C, Mead A, Li R (2003) Primordia initiation of mushroom (*Agaricus bisporus*) strains on axenic casing materials. Mycologia 95(4):620–629
2. Garland JL (1997) Analysis and interpretation of community-level physiological profiles in microbial ecology. FEMS Microbiol Ecol 24:289–300
3. Mondini C, Insam H (2003) Community level physiological profiling as a tool to evaluate compost maturity: a kinetic approach. Euro J Soil Biol 39:141–148
4. Carpenter-Boggs L, Kennedy A, Reganold JP (1998) Use of phospholipids fatty acids and C-source utilization patterns to track microbial community succession in developing compost. Appl Environ Microbiol 64: 4062–4064
5. Insam H, Feurle J, Rangger A (2001) Community level physiological profiles (Biolog substrate use tests) of environmental samples. In: Akkermans ADL, van Elsas JD, DeBruijn FJ. eds. Molecular Microbial Ecology Manual: Kluwer Academic Publishers. P. 4.1.1
6. Mondini C, Insam H (2005) Effect of Inoculum Standardization on Community Level Physiological Profiles of Compost Samples. Compost Sci Util 13(1):27–33
7. Garland JL (2000) Potential and limitations of BIOLOG for microbial community analysis. In: Bell CR, Brylinsky M, Johnson-Green P. eds. Microbial Biosystems: New Frontiers. Proceedings of the 8th International Symposium on Microbial Ecology. Atlantic Canada Society for Microbial Ecology: Halifax, Canada
8. Garland JL, Mills AL (1991) Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level-sole-carbon-source-utilization. Appl Environ Microbiol 57:2351–2359

9. Insam H, Amor K, Renner M, Crepaz C (1996) Changes in functional abilities of the microbial community during composting of manure. Microbiol Ecol 31:77–87
10. Bossio DA, Scow KM (1995) Impact of carbon and flooding on the metabolic diversity of microbial communities in soil. Appl Environ Microbiol 61:4043–4050
11. Sharma S, Rangger A, von Lutzow M, Insam H (1998) Functional diversity of soil bacterial communities increases after maize litter amendment. Eur J Soil Biol 34:53–60
12. Lawlor K, Knight BP, Barbosa-Jefferson VL, Lane PW, Lilley AK, Paton GI, McGrath SP, O'Flaherty SM, Hirsch PR (2000) Comparison of methods to investigate microbial populations in soils under different agricultural management. FEMS Microbiol Ecol 33:129–137
13. Goodfriend WL (1998) Microbial community patterns of potential substrate utilization: a comparison of salt marsh, sand dune, and seawater-irrigated agronomic systems. Soil Biol Biochem 30:8–9
14. Zak JC, Willing MR, Moorhead DL, Wildman HG (1994) Accelerated paper: Functional diversity of microbial communities: a quantitative approach. Soil Biol Biochem 26: 1101–1108
15. Wönsche L, Brüggemann L, Babel W (1995) Determination of substrate utilization patterns of soil microbial communities: an approach to assess population changes after hydrocarbon pollution. FEMS Microbiol Ecol 17:295–306
16. Gomez E, Bisaro V, Conti M (2000) Potential C-source utilization patterns of bacterial communities as influenced by clearing and land use in a vertic soil of Argentina. Appl Soil Ecol 15:273–281
17. Garland JL (1996) Analytical approaches to the characterization of samples of microbial communities using patterns of potential C source utilization. Soil Biol Biochem 28: 213–221
18. Gauch HG (1982) Multivariate Analysis in Community Ecology. Cambridge University Press: Cambridge
19. Garland JL, Mills AL (1994) A community-level physiological approach for studying microbial communities. In: Ritz K, Dighton J, Giller KE. ed. Beyond the Biomass. John Wiley and Sons: Chichester. pp. 77–83
20. Curtis TP, Sloan WT (2004) Prokaryotic diversity and its limits: Microbial community structure in nature and implications for microbial ecology. Curr Opin Microbiol 7: 221–226
21. O'Donnell AG, Goofellow M, Hawksworth DL (1995) Theoretical and practical aspects of the quantification of biodiversity among microorganisms. In: Hawksworth DL. ed. Biodiversity-Measurement and Estimation. Chapman and Hall: London, UK. pp. 65–73
22. Garcia-Pichel F, Nübel U, Muyzer G (1998) The phylogeny of unicellular, extremely halotolerant cynobacteria. Arch Microbiol 169:469–482
23. Hollibaugh JT (1994) Relationship between thymidine metabolism, bacterioplankton community metabolic capabilities, and sources of organic matter. Microb Ecol 28: 117–131
24. Jellette JF, Li WKW, Dickie PM, Boraie A, Kepkay PE (1996) Metabolic activity of bacterioplankton communities assessed by flow cytometric and single carbon substrate utilization. Mar Ecol Prog Ser 136:213–225
25. Winding AK (1994) Fingerprinting bacterial soil communities with BIOLOG microtiter plates. In: Ritz K, Dighton J, Giller KE. eds. Beyond the Biomass: Compositional and Functional analysis of soil microbial communities. Wiley: Chichester. pp. 85–94
26. Haack SK, Garchow H, Klug MJ, Forney LJ (1995) Analysis of factors affecting the accuracy, reproducibility and interpretation of microbial community C source utilization patterns. Appl Environ Microbiol 61: 1458–1468
27. Harch B.D, Corell RL, Meech W, Kirby CA, Pankhurst CE (1997) Using the Gini coefficient with BIOLOG substrate utilization data to provide an alternative quantitative measure for comparing bacterial soil communities. J Microbiol Meth 30:91–101