CULTIVABLE MICROBIOME OF FRESH WHITE BUTTON MUSHROOMS

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SIGNIFICANCE AND IMPACT OF THE STUDY

Presence and persistence of microorganisms within the microbiome of fresh produce is important when identifying a potential niche for foodborne pathogens. Most foodborne outbreaks can be attributed to microbial imbalances or lack of diversity within the associated host surface and residing microbial population. *Agaricus bisporus* samples analysed during this study showed a higher microbial load (5.2 up to 12.4 log cfu g^{-1}) compared to known values for other fresh produce. These mushrooms were considered to carry microbial loads representing a healthy and safe product, fit-for-consumption, despite showing a high indicator incidence. Although foodborne pathogens may be associated on occasion with fresh mushrooms, it remains a low risk commodity, therefore this study provides insight and experimental evidence identifying microbial population dynamics of fresh and packed mushrooms.

ABSTRACT

Microbial dynamics on commercially grown white button mushrooms is of importance in terms of food safety assurance and quality control. The purpose of this study was to establish the microbial profile of fresh white button mushrooms, with the focus on potential presence of food-borne pathogens. The total microbial load was determined through standard viable counts. Presence and isolation of gram-negative bacteria including coagulase positive Staphylococci were performed using a selective enrichment approach. Dominant and

presumptive organisms were confirmed using molecular methods. Total mushroom microbial counts ranged from 5.2 to 12.4 log cfu g^{-1}, with the genus *Pseudomonas* being most frequently isolated (45.37% of all isolations). In total, 91 different microbial species were isolated and identified using Matrix assisted laser desorption ionisation time of flight mass spectrophotometry, PCR and sequencing. Considering current food safety guidelines in South Africa for ready-to-eat fresh produce, coliform counts exceeded the guidance specifications for fresh fruit and vegetables. Based on our research and similar studies, it is proposed that specifications for microbial loads on fresh, healthy mushrooms reflect a more natural microbiome at the point-of-harvest and point-of-sale.

KEY WORDS

Food-borne pathogens; *Agaricus bisporus* microbiome; cultivable microbiome of mushrooms; *button mushroom*

Introduction

Viewed from a microbial-ecological perspective, fresh produce are commonly known to harbour a large diversity of microbial communities (Beuchat, 1996). Depending on the produce types, microbial community composition can rapidly change and be influenced by a range of environmental factors, such as temperature, pH and moisture availability. Similarly, variations in farming practices i.e. growing and storage conditions as well as transport procedures can influence the diversity and composition of the produce associated microbial communities (Leff & Fierer, 2013). Due to a high moisture content, water activity of 0.98 or higher and neutral pH (Venturini et al., 2011), mushroom surfaces provide ideal microbial niches to support microbial growth and proliferation. Regardless, the information available on the ecological diversity and microbial load of white button mushrooms are limited.

Beside the potential association of food-borne pathogenic microorganisms, it is important to understand the microbial community dynamics on mushrooms from a health and hygiene perspective. As a result of a general short shelf-life and increasing raw consumption, the indirect impact on human health due to exposure to non-pathogenic microorganisms should be considered, such as the potential of introducing new commensal bacteria into the human gastrointestinal system (Leff & Fierer, 2013). The cultivation dynamics, harvesting, handling

and storage methods used during mushroom production differ from that of other fresh produce. Therefore, microbial population profiles and -loads found on fresh mushrooms should be considered separately.

This study was aimed at determining the microbiome dynamics of freshly harvested and packaged white button mushrooms, collected from two large scale commercial farms over a two-year-period. Total microbial loads were determined at the point-of-harvest as well as after packing, before transport to retailers and to ultimately serve as a guideline for safe mushrooms. In addition, the presence or absence of Thermotolerant coliform bacteria on fresh mushrooms were analysed to determine compliance with national guidance documents (Department of Health, 2000) and to provide a scientific based mushroom assessment.

Results and Discussion

Microbial load assessment on mushrooms

Bacterial counts ranged from 2.5 to 6.2 log cfu g^{-1}, fungal counts between 0.0 and 2.7 log cfu g-1 and yeasts from 1.0 to 4.3 log cfu g^{-1}. Average total culturable microbial counts ranged from 5.2 (min) to 12.4 (max) log cfu g^{-1}, with the average total microbial count being 8.7 log cfu g^{-1} (Table 1). At the point of harvest, fresh first break mushrooms showed a total microbial count of 5.2 – 10.1 log cfu g^{-1}, and when packed 8.0 – 10.1 log cfu g^{-1}. Similarly, total microbial loads for second and third break mushrooms at harvest were determined to be 7.6 – 10.2 log cfu g^{-1} and 8.8 – 11.5 log cfu g^{-1} respectively and correspondingly, 8.3 – 10.5 log cfu g^{-1} and 9.5 – 12.4 log cfu g^{-1} when packed. When viewed in the context of the interactive effect that exists between mushroom production factors on the dependant measure (growth cycle - break; farm sampled on; room sampled from; production phase - at harvest or packed) it is statistically shown that the impact of one factor is reliant on the impact of the other.

The mushrooms investigated in this study reflected a relatively high total microbial load when compared to other studies. Reyes *et al*. (2004) reported mesophilic aerobic loads of 7.9 log cfu g^{-1} in a study to determine the prevalence of *Enterobacteriaceae* species specifically *E. americana* and the pathogenic potential of the isolated strains on commercial packed/retail mushrooms. Venturini *et al*. (2011) indicated that the total microbial load on *A. bisporus* from packed/retail samples ranged between 6 and 8 log cfu g^{-1} in a study to determine the safety and quality of various mushroom species. A recent study by Siyoum *et al.* (2015) described microbial

Table 1: *Bacteria, yeast, fungal and thermotolerant coliform counts on fresh white button mushrooms*

			Microbial counts (log cfu g-1) mean ± standard deviation |[a]							
			Farm 1				Farm 2			
	Production phase	Production cycle (Break)	Bacteria	Thermotolerant coliforms	Yeasts	Fungi	Bacteria	Thermotolerant coliforms	Yeasts	Fungi
Room 1	Pre-harvest	1st	2.5 ± 2.7 |G	2.5 ± 0.3 |EFGH	1.0 ± 1.9 |G	1.7 ± 1.1 |BC	5.6 ± 0.7 |ABCDE	2.7 ± 0.3 |DEFG	3.5 ± 0.5 |ABCD	ND
		2nd	4.9 ± 0.2 |DEF	2.8 ± 0.3 |CDEFG	1.8 ± 1.1 |FG	1.6 ± 0.9 |BCD	6.2 ± 0.6 |A	2.8 ± 0.3 |DEFG	4.1 ± 0.5 |A	ND
		3rd	5.4 ± 0.3 |ABCDEF	2.9 ± 0.2 |CDEF	4.0 ± 0.4 |AB	1.8 ± 0.3 |B	5.9 ± 0.5 |AB	2.8 ± 0.2 |CDEFG	3.3 ± 1.4 |ABCD	ND
	Post-packaging	1st	4.7 ± 1.9 |F	2.6 ± 0.2 |DEFG	2.9 ± 2.4 |CDEF	0.4 ± 0.8 |EF	5.7 ± 0.8 |ABCD	2.4 ± 0.6 |GHI	3.8 ± 0.6 |ABC	ND
		2nd	4.8 ± 0.3 |EF	2.7 ± 0.2 |DEFG	2.5 ± 1.4 |DEF	1.9 ± 0.8 |B	5.6 ± 0.4 |ABCDEF	2.8 ± 0.4 |DEFG	3.6 ± 0.6 |ABC	ND
		3rd	4.9 ± 0.3 |CDEF	2.8 ± 0.2 |CDEFG	3.2 ± 0.6 |ABCD	1.7 ± 0.2 |B	5.5 ± 2.3 |ABCDEF	3.0 ± 0.5 |CDE	3.9 ± 0.6 |AB	ND
Room 2	Pre-harvest	1st	5.4 ± 0.8 |ABCDEF	3.1 ± 0.1 |BCD	2.9 ± 0.9 |BCDE	0.9 ± 1.3 |DE	5.9 ± 0.4 |ABC	2.5 ± 0.4 |FGH	3.8 ± 0.6 |ABC	0.5 ± 1.3 |EF
		2nd	5.2 ± 0.4 |BCDEF	3.6 ± 0.5 |AB	3.4 ± 0.7 |ABCD	1.9 ± 0.8 |B	5.9 ± 0.4 |ABC	2.7 ± 0.3 |DEFG	3.7 ± 1.6 |ABC	ND
		3rd	5.7 ± 0.4 |ABCD	3.7 ± 0.4 |A	4.3 ± 0.4 |A	1.5 ± 1.1 |BCD	6.1 ± 0.6 |A	2.9 ± 0.3 |CDEFG	3.8 ± 0.3 |ABC	ND
	Post-packaging	1st	5.5 ± 0.5 |ABCDEF	2.9 ± 0.2 |CDEF	2.5 ± 1.6 |DEF	1.0 ± 1.1 |CDE	5.9 ± 0.6 |ABC	1.9 ± 1.2 |I	3.8 ± 0.4 |ABC	ND
		2nd	5.1 ± 0.4 |BCDEF	3.1 ± 0.3 |BCD	3.2 ± 0.6 |ABCD	1.9 ± 0.5 |B	5.6 ± 0.3 |ABCDEF	1.9 ± 1.2 |I	2.0 ± 2.2 |EFG	ND
		3rd	5.6 ± 0.3 |ABCDEF	3.3 ± 0.3 |ABC	4.2 ± 0.6 |A	2.7 ± 0.7 |A	5.9 ± 0.4 |AB	2.0 ± 1.3 |HI	3.8 ± 0.3 |ABC	ND

Total (average) microbial load: **8.7 ± 0.1 log cfu g^{-1}**

Total microbial load range (min-max): **5.2 - 12.4 log cfu g^{-1}**

[a] For each column, mean values followed by statistical t-grouping according to analysis with a two way-ANOVA showing interaction effect between farms, pre-harvest, post-packaging, rooms and production cycles (breaks). Same letter/combination of letters in each column do not differ significantly from each other at a $p < 0.05$ significance level.

ND: not detected, counts below detection limit.

succession on healthy mushrooms at the point of harvest, which represented a 4 to 5 log cfu g^{-1} representative of a climax bacterial community. Microbial loads could according to the previous authors reportedly increase to log 7 to 9 cfu g^{-1} depending on the storage conditions. It can be deduced that a healthy, safe mushroom can carry a total microbial load ranging between 5.2 log cfu g^{-1} and 12.4 log cfu g^{-1}, depending on the relation between farm, production phase, and -break as well as growing room conditions from which the mushrooms are harvested.

Microbial dynamics and succession between different production stages

Variable bacterial counts were observed during the progression from first to third break and between each of the five rooms sampled. First break bacterial counts ranged from 5.4 to 6.2 log cfu g^{-1} for pre-harvest and 5.6 to 6.1 log cfu g^{-1} packaged mushrooms. Bacterial counts during second break fluctuated between 5.7 and 6.0 log cfu g^{-1} for pre-harvest and 5.7 to 6.2 log cfu g^{-1} for packaged mushrooms. Third break bacterial counts ranged from 5.6 to 6.1 log cfu g^{-1} for pre-harvest mushrooms and 5.9 to 6.1 log cfu g^{-1} for packaged mushrooms. The mean counts being 5.8 log cfu g^{-1} for both the pre-harvest and packaged mushroom samples. In a study performed by Doores *et al*. (1987), bacterial counts were determined between different breaks of pre-harvested white button mushrooms, the authors reported counts from 6.3 to 7.2 log cfu g^{-1}. Therefore, microbial loads found in this study were thus higher at post-packaging compared to the at-harvest stage. Load differences between the former and latter were however not significant, which means that microbial loads reflected by packaged mushrooms are comparable to loads found on mushrooms at the "point-of-harvest". Microbial loads found on fresh mushrooms showed similarities and were comparable between the different growing rooms studied.

The determined thermotolerant coliform load ranged from 2.54 – 3.71 log cfu g^{-1} (Table 1). No correlation could be found with reference to the progression of coliform load from first to third break. Farm 1 showed a higher coliform load throughout the production cycles, and between pre-harvest and packaged samples within each cycle in contrast to Farm 2 (Table 1). Low counts of thermotolerant coliform bacteria and the absence of gram-*negative bacteria* were similarly described by Gónzalez-Fandos *et al*. (2000). Yeast counts of pre-harvest and packaged mushrooms showed variable progression over time for each of the five rooms sampled. First break yeast counts were found to be in the range of 2.8 to 3.9 log cfu g^{-1} for pre-harvest and 2.9 to 4.0 log cfu g^{-1} for packaged mushrooms (Table 1). During the second break, yeast counts showed a range varying from 2.3 to 3.9 log cfu g^{-1} for pre-harvest mushrooms and 3.5 to 3.9 log cfu g^{-1} for packaged mushrooms. Third break yeast counts ranged from 2.3 to 4.2 log cfu g^{-1} for pre-harvest mushrooms and 3.6 to 4.2 log cfu g^{-1} for packaged

Table 2: *Most frequently isolated bacterial species from white button mushrooms, during different production stages, identified using the Matrix Assisted Laser Desorption Ionisation-Time Of Flight (MALDI-TOF MS)*

MALDI-TOF isolate identification	Percentage (%) occurrence	MALDI-TOF MS Identification accuracy (3.00 = 100% Accurate)	16S BLAST Result (Confirmed species)	Similarity Score %	Pre- / Post-harvest isolation
Pseudomonas extremorientalis	12.20	2.07 ± 0.07	*Pseudomonas extremorientalis*	95	48% Pre / 52% Post
Raoultella ornithinolytica	10.52	2.43 ± 0.12			41% Pre / 59% Post
Microbacterium oxydans	9.86	2.06 ± 0.15	*Microbacterium oxydans*	96	22% Pre / 78% Post
Enterobacter cloacae	5.68	2.27 ± 0.13	*Enterobacter cloacae*	89	35% Pre / 65% Post
Pseudomonas tolaasii	5.35	2.23 ± 0.11	*Pseudomonas tolaasii*	99	32% Pre / 68% Post
Pseudomonas orientalis	5.02	1.87 ± 0.07	*Pseudomonas orientalis*	97	23% Pre / 77% Post
Microbacterium maritypicum	4.18	2.05 ± 0.17	*Microbacterium maritypicum*	96	14% Pre / 86% Post
Pseudomonas rhodesiae	4.01	1.94 ± 0.09	*Pseudomonas rhodesiae*	98	25% Pre / 75% Post
Staphylococcus succinus	3.84	1.77 ± 0.09	*Staphylococcus succinus*	98	13% Pre / 87% Post
Pseudomonas antarctica	3.67	2.04 ± 0.07	*Pseudomonas antarctica*	97	41% Pre / 59% Post
Pseudomonas kilonensis	3.67	1.85 ± 0.08	*Pseudomonas kilonensis*	97	18% Pre / 82% Post
Pseudomonas chlororaphis	3.34	1.87 ± 0.08	*Pseudomonas chlororaphis*	98	30% Pre / 70% Post
Microbacterium liquefaciens	2.83	1.91 ± 0.12	*Microbacterium liquefaciens*	90	18% Pre / 82% Post
Enterobacter asburiae	2.50	2.22 ± 0.16	*Enterobacter asburiae*	98	13% Pre / 87% Post
Pseudomonas azotoformans	2.50	2.03 ± 0.06	*Pseudomonas azotoformans*	97	33% Pre / 67% Post
Pseudomonas frederiksbergensis	2.34	1.93 ± 0.09	*Pseudomonas frederiksbergensis*	96	36% Pre / 64% Post
Citrobacter freundii	2.17	2.32 ± 0.17			54% Pre / 46% Post
Staphylococcus equorum	2.17	1.99 ± 0.11	*Staphylococcus equorum*	99	0% Pre / 100% Post
Serratia marcescens	2.17	2.26 ± 0.14	*Serratia marcescens*	99	46% Pre / 54% Post
Pseudomonas marginalis	1.99	1.99 ± 0.11	*Pseudomonas marginalis*	97	17% Pre / 83% Post
Salmonella spp.	1.99	2.39 ± 0.12	*Salmonella enterica*	97	50% Pre / 50% Post
Cedecea neteri	1.83	2.04 ± 0.15	*Cedecea neteri*	97	55% Pre / 45% Post
Citrobacter braakii	1.83	2.41 ± 0.10			9% Pre / 91% Post
Pseudomonas mandelii	1.50	2.12 ± 0.07	*Pseudomonas mandelii*	99	44% Pre / 56% Post
Staphylococcus saprophyticus	1.49	1.89 ± 0.12	*Staphylococcus saprophyticus*	74	44% Pre / 56% Post
Staphylococcus xylosus	1.34	2.04 ± 0.29	*Staphylococcus xylosus*	99	63% Pre / 37% Post

mushrooms. The mean yeast counts across the different breaks were 3.5 log cfu g^{-1} for pre-harvest and 3.7 log cfu g^{-1} for packaged mushrooms. Bacterial, yeast and coliform counts differed significantly between mushroom growing rooms. The microbial diversity observed in this study showed a larger variety of bacteria compared to yeast and fungi (Table 2). In the study by Siyoum *et al.* (2015), the authors described a similar trend of fungi and yeasts having a lower presence and fungal diversity (dominated by *Penicillium spp.*) while bacteria were dominant and showed diversity. Dissimilarly, to both our study and that by Siyoum *et al.* (2015), Chikthimmah (2006) described larger fungal loads of ~6 log cfu g^{-1} on freshly harvested mushrooms in contrast to yeasts and bacteria. Very little information is available for yeasts associated with mushrooms as well as the role it plays in spoilage of the final food product. The most frequently isolated and identified yeasts and bacteria made up 81.55% of the total population, with the most dominant yeast identified as *Rhodotorula mucilaginosa* (Table 3). A total of five different fungal species were identified of which *Penicillium brevicompactum* (Dierckx, 1901) were found to be dominant (66.67% of all fungal isolates). *Trichoderma longibrachiatum* (Rifai, 1969) represented 13.33% of the fungal species in contrast to *Penicillium toxicarium* (Miyake, 1940), *Cladosporium cladosporioides* (Fresen.) (G.A. de Vries, 1952), *Didymella fabae* (G.J. Jellis and Punith, 1991) each representing 6.67% of the total fungal population isolated and identified from white button mushrooms. (Table 3).

Various species of *Pseudomonas* were isolated from mushrooms throughout this study. The genus *Psuedomonas* has been well described as one of the most important groups of bacteria present on pre-harvest and packaged mushrooms (Siyoum *et al*., 2015). It has also been shown that *A. bisporus* has the highest *Pseudomonas* counts in comparison to other mushroom species (Venturini *et al*., 2011). Interactions of some pseudomonads such as *P. tolaasi, P. fluorescens* and *P. putida* (all isolated in this study) can have beneficial or negative effects for mushroom production or disease control (Frey-Klett *et al*., 2011). *Pseudomonas putida*, for instance is best known for its essential role in stimulating and contributing to mushroom fruit body formation in earlier stages of mushroom production (Noble *et al*., 2009). It has been shown that, some pseudomonads as well as other non-pathogenic bacteria play an important role in the eventual spoilage and quality degradation of harvested mushrooms. The majority of the bacteria isolated were mainly Gram-negative, including members of the family *Enterobacteriaceae*. Some of these organisms are considered indicators of potential faecal contamination in foods. However, their presence cannot automatically be associated with the incidence of enteropathogens.

Table 3: *Most frequently isolated yeast and fungal species from white button mushrooms, different production stages, identified through the use of ITS sequencing and BLAST analysis*

ITS BLAST Result (Confirmed species)	Percentage (%) occurrence	Similarity Score %	Pre- / Post-harvest isolation
Yeast			
Rhodotorula mucilaginosa	84.31	97	100% Post
Cystofilobasidium infirmominiatum	14.02	98	34% Pre / 66% Post
Candida spp.	1.67	98	78% Pre / 22% Post
Fungi			
Penicillium brevicompactum	66.67	99	40% Pre / 60% Post
Trichoderma longibrachiatum	13.33	99	50% Pre / 50% Post
Penicillium toxicarium	6.67	99	100% Post
Cladosporium cladosporioides	6.67	99	100% Post
Didymella fabae	6.67	99	100% Post

Although association of potential human pathogens have been confirmed in this study, it is unlikely that a single strategy will be successful in eliminating contamination of freshly cultivated mushrooms. A multi-pronged approach may be considered by regulatory authorities, retail and the industry based on effective implementation of a science based regulation. This should be supported by effective enforcement and good agricultural practices in the commercial mushroom industry. Furthermore, adhering to good manufacturing practices during packaging or minimal processing, storage and distribution may reduce the hazards of food-borne related diseases. Similar integrated management models have been implemented successfully in alternative fresh produce production systems and it is likely that such an approach will also work for risk minimization of human pathogenic bacteria on fresh white button mushrooms.

Materials and methods

Sample collection

Two experimental designs were employed to assess the microbial dynamics of mushrooms during different production phases. In order to determine microbial shifts on mushrooms between production and harvesting/packaging (Experiment 1), a total of 432 samples, consisting of 250g commercial punnet units (12-16 mushrooms per unit depending on size), were collected before harvest and after packaging. Sampling was done during three stages of production (1st, 2nd and 3rd breaks). Mushroom samples were selected at the commercially ready-to-harvest stage, based on uniformity of shape, size, and maturity as well as being devoid of mechanical defects or browning according to the national commercial standard for white button mushrooms (DAFF, Agricultural Products Standards Act, No. 119 of 1990, Regulation No. R.364). Eight samples were picked aseptically before harvest from eight growing beds (mushrooms pooled from a single growing bed, constitutes one sample) and placed into punnets. To further determine population shifts of culturable microorganisms, mushrooms were monitored for a 12-week-period from full bud growth through harvesting, packing up to cold storage (3°C ± 2°C). A total of 192 samples were collected in 12 different mushroom growing rooms, using a completely randomised design. To determine microbial succession within growing cycles i.e. from first break until the third break, five individual mushroom growing rooms were sampled (Experiment 2). In total, 240 samples were procured and analysed over a 15-week-period. A systematic sampling design was used and the experiment was repeated on each farm. The farms were located in the same province and followed similar

production practices. Both farms are considered large commercial scale production units certified to the GlobalG.A.P. Integrated farm assurance standard.

Microbiological analysis

Each sample (250g) was placed within an aseptic (75% ethanol rinsed and air-dried) stainless-steel beaker. The mushrooms were blended using a handheld, ethanol cleaned food-blender. From the blended sample, 10 g were weighed off into a sterile stomacher bag containing 90 ml sterilised tryptone soy broth (Merck-Biolab, Johannesburg, South Africa). Contents of the bag were homogenised in a Stomacher® Circulator 400 (5 min at 230 rpm). Standard serial dilutions were made using buffered peptone water (Merck-Biolab) and aliquots were spread-plated onto standard one agar for bacteria and malt extract agar for yeasts and moulds (Merck-Biolab). All colonies were counted to determine total viable counts and dominant and representative colonies were isolated for further confirmation of identity. Colonies were purified and preserved at -70°C using glycerol solutions of 10% and 55% respectively for fungi, yeasts and bacteria (Hubalek, 2003).

Identification of bacterial, yeast and mould isolates

Purified cultures of representative bacterial colonies were transferred in duplicate directly to a MALDI-TOF target plate (Bruker), and overlaid with the α-cyano-4-hydroxycinnamic acid matrix (Bruker). The plate was subsequently analysed using BrukerMicroFlex LT MALDI-TOF in conjunction with BrukerBiotyper automation software and library. The MALDI-TOF was calibrated prior to use with the bacterial standard according to supplier guidelines. Duplicate score values (SV) were recorded; SV were used to determine the accuracy of identification. A SV of between 1.999 and 1.700 was used to identify the genus name of the organism, and a value of above 2.0 was used to determine the genus and probable species of an organism. A SV within the range of 2.300 – 3.000 were considered as conclusive species identification.

Using the MALDI-TOF in this type of application as a novel technology, verification and confirmation of results were performed through species specific regions of ITS rDNA or 16S sequencing of all yeasts and moulds specifically bacterial isolates that could not be accurately identified with the MALDI-TOF (Log Score Value <2.3). DNA was extracted using the Quick-GDNA miniprep kit (ZymoResearch) for bacteria and Nucleospin® Plant II DNA extraction kit (Macherey-Nagel) for the yeasts and moulds. Depending on the organism either 16S primers (27 Forward 5'- GAGTTTGATCCTGGCTCAG-3' / 1492 Reverse 5'-TACGGYTACCTTGTTACGACTT-3') (Weisburg *et al.*, 1991) or ITS primers (1 Forward 5'-

TCCGTAGGTGAACCTGCGG-3' / 4 Reverse 5'- TCCTCCGCTTATTGATATGC-3') (White *et al*., 1990) were used for sequence amplification. PCR reactions were conducted in 100µl thin-walled tubes using an Eppendorf 6325 Mastercycler Pro S 96 well Thermal Cycler Vapo Protect (Eppendorf). Each 25 µl bacterial reaction mixture contained 0.3 µl MyTaq™ DNA Polymerase (Bioline), 5µl MyTaq™ Reaction Buffer (Biolone), 0.3 µl PCR Forward Primer (IDT, South Africa), 0.3 µl PCR Reverse Primer (IDT), 1 µl DNA Template and 16.3 µl H_2O (sterile distilled water). Each 25 µl fungal and yeast reaction mixture contained 0.25 µl MyTaq™ DNA Polymerase (Bioline), 5.25 µl MyTaq™ Reaction Buffer (Biolone), 0.25 µl PCR Forward Primer (IDT, South Africa), 0.25 µl PCR Reverse Primer (IDT), 1 µl DNA Template and 18.8 µl H_2O (sterile distilled water). Thermal cycling for bacterial, fungal and yeast reactions consisted of an initial denaturation at 95°C for 2 min. followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 45 s, and extension at 72°C for 1 min. 30 s. The PCR products were visualised following gel electrophoresis on 1% agarose gel for 16S amplicons and a 2% agarose gel for ITS amplicons stained with 0.01 % ethidium bromide in a Vilber Lourmat (Omni-Science CC, South Africa) gel imaging system. The desired bands were cut from the gel and purified (Zymoclean™ Gel DNA Recovery Kit - Zymo Research), sequenced and analysed by Inqaba Biotech™ (Pretoria). Lastly, a BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) search was carried out for each gene sequence using the NCBI gene bank database (Pruitt *et al*., 2005).

Statistical analysis

Both random as well as random-systematic sampling were utilised for the collection of mushroom samples during this study. Microbiological count data were analysed by two-way analysis of variance General Linear Model (GLM) test (SAS software version 9.3). Interaction effect was analysed between farms, pre-harvest, post-packaging, rooms and production cycles (breaks). There was strong evidence for a four factor interaction. A significance level (p-value) of <0.05 was used as the decisive criteria for significant differences. The Fisher Least Significance Difference (LSD) test was used to investigate the nature of the differences.

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Conflict of Interest

No conflict of interests were identified.

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