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Full Length Research Article

BACTERIA ASSOCIATED WITH CULTURES OF *PSATHYRELLA ATROUMBONATA* (Pegler) AND *SCHIZOPHYLLUM COMMUNE* (Fr. Ex. Fr.)

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Studies were carried out on bacteria, which usually infect spawns and culture plates of Psathyrella atroumbonata (Pegler) and Schizophyllum commune (Fr. Ex. Fr.), two Nigerian edible mushrooms. During the vegetative propagation of these higher fungi, six different bacteria species were isolated and characterized from 14 day old spawns and mycelial ramified PDA culture plates. These bacteria include Bacillus licheniformis, Bacillus subtilis, Leuconostoc mesenteroides, Pseudomonas aeruginosa, Bacillus cereus and Staphylococcus aureus. The average bacteria count was 1.0 x 10^6 cfu/ml and these bacteria grew within pH range of 5.0 and 9.0. the optimum temperature range of growth lied between 30^0C and 37^0C. The significance of these findings to the cultivation of P. atroumbonata and S. commune in Nigeria were discussed.

Key words: *Physiological studies, bacteria, cultures, mushrooms, spawn*

INTRODUCTION

In many countries of the world including Nigeria, edible mushrooms have been priced as delicacies for several years. Apart from their medicinal values, they constitute an important food source in a world that is threatened by energy food crisis. Mushrooms have been reported to be rich in protein, glycogen, vitamins, crude fibres and essential mineral compounds (Ogundana and Fagade, 1982; Fasidi, 1996; Jonathan, 2002; Stamets, 1993). Infact, Bano (1976) reported that nutrient contents of mushrooms lied between meat and vegetables.

Mushrooms such as *Flammulina velutipes, Lentinus edodes, Agaricus bisporus, Pleurotus oestratus, Volvariella volvacea* and *Agaricus campestris* among others, have been cultivated for food in several countries of the world especially in America, Europe and few Asian countries. Artificial cultivation of mushrooms is new to many developing countries and there is a death of information on fundamental processes governing their growth. Because of this reason, people in Nigeria depends mainly mushrooms that are obtained from the wild.

Spawns and composts used for mushroom growing are nutritionally and biologically active to support the growth of mushrooms and other unwanted microorganisms. Therefore, preparing mushroom composts, spawns and plates require skills and experience. This is because mushroom growing processes are always been intruded and spoilt by bacteria, fungi and nematodes leading to low yield and thereby causing economic loss for mushroom growers.

Presently, the identity of bacteria contaminants of mushroom spawns and culture plates in Nigeria has not been well documented. Therefore, the objective of this study was to isolate and characterize such bacteria and make suggestions on how they could be controlled.

MATERIAL AND METHODS

Sample Preparation

Rice straw spawns were prepared in 350ml jam bottles using the method of Fasidi and Ekuere (1993). The bottles after being filled with substrates were covered with aluminum foil and autoclaved at 1.02/kgcm^{-2} pressure for 20 minutes. After cooling, the

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bottles were inoculated with 5 – day old (actively growing) mycelial discs of *P. atroumbonata* and *S. commune* and incubated at 30 $\pm$ 2^0C for 21 days. The bacteria infected spawns were assessed visually for colony formation and selected for microbial analyses.

For culture plates, healthy fruit bodies of *P. atroumbonata* and *S. commune* were tissue –cultured to obtain mycelia using the method described by Jonathan and Fasidi (2001). The pure mycelial cultures of these fungi were maintained on plates of PDA supplemented with 0.5% yeast extract. The plates were assessed visually for colony formation after 4 days to verify contamination of mushroom spawn from unwanted bacteria.

Isolation of Bacteria

Ten grammes (10.0g) each of infected spawns of *P. atroumbonata* and *S. commune* were separately soaked in 90.0cm^3 of sterile de-ionised water in 250cm^3 conical flasks. The mixture was shaken properly and 1.0cm^3 of it was transferred into a sterile McCartney bottle containing 9.0cm^3 of sterile de-ionised water. This process was repeated for other sterile McCartney bottles so that at the end, dilution of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} folds were obtained. Each dilution was then plated out by spread plate method on plate count agar (PCA) (Oxoid)and nutrient agar (NA) (Oxoid) (Okafor, 1977).

A standard plate count was carried out on colonies obtained on solid PCA using electronic Gallenkamp model colony counter and the unit expressed in cfu/ml. All the bacterial isolates were streaked separately onto nutrient agar and, Eosine methylene blue agar (EMB) plates to obtain pure cultures and determine their colonial morphology respectively. For agar plates that contained mycelia of *P. atroumbonata* and *S. commune*, the bacteria infected plates were also streaked onto a new petri dishes containing sterile NA and EMB. They were incubated at 35^0C for 48 hours. The bacteria isolated from spawns were subjected to Gram's reaction, motility, catalase, oxidase, indole and other standard biochemical tests shown on Table 3 (Prescott *et al*, 1993. Bacterial identification and characterization were carried out by comparing the results obtained with the characterization definitions of Skinner and Lovelock (1979) and that of Bergey's'Manual of systematic determinative bacteriology (2001).

Effect of Temperature on Growth of Bacteria Isolates

Five milliliters of nutrient broth (NB0 (Oxoid) were dispensed into each of the 27 screw capped test tubes. They were autoclaved at 1.02kgcm^{-2} pressure for 15 minutes. After cooling, the test tubes were inoculated with 0.1ml of different bacterial isolates and incubated at 10, 20, 25, 30, 35, 37, 40 and 45^0C respectively. Each treatment was replicated thrice. The control tubes were left without any inoculum. Growth was measured after 48 hours at 540nm using a Pye unicam spectrophotometer.

Effect of pH on Growth of Bacteria Isolates

Citrate-Phosphate buffer solutions of pH 5.0, 6.0, 7.0, 8.0 and 9.0 were prepared (Ekunsanmi and Arojojoye 1997). Double strength nutrient broth (20.0cm^3) was added to equal quantity of each buffer solution in separate 150.0cm^3 conical flask to give 40.0cm^3 nutrient broth of normal strength. Three replicates of these were prepared for each pH and the bottles were autoclaved at 1.02kgcm^{-2} pressure for 15 minutes.

After cooling, the flasks were singly inoculated with the bacterial isolates and incubated at 35^0C for 24 hours. Growth was assessed by turbidity after 48 hours using a Pye unicam Spectrophotometer at 540nm wavelength. A graph of growth against time was then plotted for the different pH levels.

RESULTS AND DISCUSSION

Six different bacteria species were isolated from mushroom spawns and culture plates used in these studies. The isolates consisted of one Gram-negative bacteria (*Pseudomonas aeruginosa* BI_3) and five Gram positive bacteria (*Leuconostoc mesenteroides* BI_1; *Bacillus subtilis,* BI_2; *Staphylococcus aureus,* BI_4; *Bacillus cereus,* BI_5; and *Bacillus licheniformis,* BI_6). They were identified by a series of morphological, biochemical as well as physiological characteristics using the standard characterization procedure of Skinner and Lovelock (1979) and that of Bergey's Manual of systematic bacteriology (2001).

The frequency of occurrence of each bacteria isolate is represented on Table 1. *Bacillus subtilis* BI_2 was isolated in all the five samples. *Staphylococcus aureus* BI_4 was recorded four times while *B.licheniforms* BI_6 was isolated three times (Table 1). The frequency of occurrence of *B.subtilis* is not a surprise because Corlett and Brown (1980)

implicated this organism as one of the major bacterial contaminants of laboratory cultures. This organism has also been linked with the spoilage of various foods such as bread, fish, milk, soymilk and mayonnaise (Akinrele, 1984).

Table 1:
Percentage of Occurrence of Microbial Isolates From Five Isolation

Bacterial Isolates	MU	N.R	% occ.
B_1	PCA	2	40%
B_2	PCA	5	100%
B_3	PCA	2	40%
B_4	PCA	4	80%
B_5	PCA	1	20%
B_6	PCA	3	60%

Legend: MU = Medium used, NR = Number of times an organism is recorded. % 0cc. = Percentage of occurrence PCA = Plate Count Agar.

Isolate BI_2 was characterised as *B.subtilis* because it was Gram positive bacillus with central spores. The organism reduced nitrate, hydrolysed starch, gelatin and casein (Table 3). Morphologically, it formed creamy, circular and translucent colonies with 1.2mm diameter and its consistency was friable (Table 1). This result agrees with the standard descriptions of Prescott *et al* (1993). A simplified classification based on morphology, endospore formation, Voges proskuer test, gelating hydrolysis, indole production and sugar fermentation tests were used to classify mesophillic spore formers as *B.cereus* (Isolate BI_5) and *B.lichinifomis (*BI_6) respectively (Table 3). These properties agree with the standard characterization procedure of Bergey's Manual of determinative bacteriology (2001). Bacilli genera were also linked with the spoilage of a Nigerian local snack "(dodo ikire)" by Jonathan and Olowolafe (2001), and fermentation of oil been seeds by Kolawola and Okonkwo (1985).

Isolate BI_2 was characterized as *L.mesenteroides* (Table 2 and 3) because of its tiny colonies, dull dried surfaces, non-motility, hydrolysis of casein and its reaction on catalase and oxidase was negative. This organisms has been implicated in the spoilage of castol oil and Pineaple (Barber *et al* 1987). Bacterial isolate BI_4 was identified as *S. aureus.* The cells of this organism were Gram-positive cocci in clusters. It fermented mannitol strongly with gas production and the coagulase test was positive. This bacteria could have been introduced into the mushroom spawns after sterilization during inoculum. The inability of BI_3 to ferment most sugars, ability to hydrolyse starch and its motility polar flagellation were among the characteristics used to classify it as *P. aeruginosa.* Similar observation was made by Jonathan and Olowolafe (2001) who reported that *P.aeruginosa* can oxidize most sugars but cannot ferment them. The complete absence of coliforms from the mushroom spawns and culture plates indicate the absence of feacal contamination (Brock et al, 1986).

Table 2: Colonial morphology of the isolates

	BI_1	BI_2	BI_3	BI_4	BI_5	BI_6
Medium	PCA	PCA	PCA	PCA	PCA	PCA
Colour	Off white	Creamy	Off white	Light yellow	Creamy	Creamy
Shape	Spherical	Circular	Spherical	Circular	Circular	Spherical
Size	1.0mm	1.2mm	1.7mm	0.9mm	1.5mm	1.6mm
Surface	Dull and dry	Dull and rough	Dull	Dull and rough	Dull	Dull and dry
Fdge	Entire	Rhizoid	Entire	Undulate	Rhizoid	Entire
Opacity	Opaque	Translucent	Opaque	Opaque	Opaque	Opaque
Degree of Growth	Moderate	Profuse	Scantly	Profuse	Moderate	Moderate
Elevation	Flat	Low convex	Plateaux	Flat	Low	Flat
Consistency	Friable	Friable	Friable	Friable	Friable	Friable
Emusifiability	Easy	Easy	Easy	Easy	Easy	Easy

Table 3: Microscopic and Biochemical Characteristics of Isolated Bacteria

	BI_1	BI_2	BI_3	BI_4	BI_5	BI_6
Cell shape	Spherical in pairs or clumps	Fat rod in singles or short chain	Small slender rods in pairs or short chain	Spherical and in clumps	Short rod in chins mostly	Small rods in pairs or clumps
Gram Reaction	+Ve	+Ve	-Ve	+Ve	+Ve	+Ve
Endo spore formation	-Ve	+Ve	-Ve	-Ve	+Ve	+Ve
Spore position	No spore	Central	No Spore	No Spore	Central	Central
Motility	-Ve	+Ve	+Ve	-Ve	+Ve	+Ve
Catalase	-Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Oxidase	+Ve	-Ve	+Ve	-Ve	-Ve	+Ve
Indole reaction	-Ve	-Ve	-Ve	-Ve	+Ve	+Ve
Gelatin hydrolysis	-Ve	+Ve	-Ve	-Ve	+Ve	+Ve
Methyl red test	+Ve	+Ve	-Ve	-Ve	+Ve	-Ve
Vogos Proskauer text	+Ve	+Ve	-Ve	-Ve	+Ve	-Ve
Casein hydrolysis	+Ve	+Ve	+Ve	-Ve	+Ve	+Ve
Starch hydrolysis	-Ve	+Ve	+Ve	-Ve	+Ve	+Ve
Nitrate reduction	-Ve	+Ve	-Ve	+Ve	+Ve	+Ve
Coagulase test	-Ve	-Ve	-Ve	+Ve	-Ve	-Ve
Urease test	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
Glucose	A	A	-Ve	AG	A	AG
Fructose	-Ve	A	-Ve	A	A	AW
Galactose	A	-Ve	-Ve	A	A	-Ve
Sucrose	AW	A	-Ve	A	A	AG
Maltose	-Ve	A	-Ve	A	AW	A
Lactose	AG	AW	-Ve	A	A	-Ve
Raffinose	-Ve	A	-Ve	A	-Ve	A
Mannitol	-Ve	AW	AW	AG	A	A
Propable	*Leuconostoc*	*Bacillus*	*Pseudomonas*	*Staphylococcus*	*Bacillus*	*Bacillus*
Identity	*Mesenteroides*	*Subtilis*	*Aeruginosa*	*Aureus*	*Cereus*	*Licheniformis*

Legend: -Ve = Negative reaction, +Ve = positive reaction, AG= Acid with gas production, A= Acid production only, AW = Weak acid production.

Table 4: Effect of Temperature on Growth of Bacteria Isolates

	OPTICAL DENSITY OF BACTERIA ISOLATES AT 540nm					
Temperature (^{0}C)	*L. mesenteroides*	*B. subtilis*	*P. aeruginosa*	*S. aureus*	*B. cereus*	*B. licheniformis*
10	1.25e	0.75d	1.87c	0.61	1.1ef	1.85d
20	1.47de	2.00bc	2.83b	2.00d	2.00d	2.25d
25	2.00cd	2.79b	3.00b	2.65cd	2.50cd	3.58c
30	2.50c	2.64b	3.08b	2.93c	3.00bc	4.25ab
35	4.25a	4.30a	4.15a	4.00b	3.63ab	4.50a
37	3.50b	3.85a	4.50a	4.92a	4.00a	3.75bc
40	1.35e	1.75c	1.83c	2.10d	1.90de	1.23e
45	1.00e	1.42cd	0.70d	1.00e	0.47f	0.85e
LSD (0.05)	0.75	0.82	0.67	0.78	0.83	0.55

Temperature had significant effect on growth of bacterial isolates (Table 4). *Leuconostoc mesenteroides, B. subtillis* and *B. licheniformis* had their optimum temperature of growth at 35^{0}C.This result agrees with the suggestion of Brock et al (1986) that these microorganisms are mesophiles. *Pseudomonas aeruginosa, S.aureus* and *B.cereus* grew best at 37^{0}C (Table 4). The ability of these bacteria, to grow optimally at 37^{0}C probably enables them to survive well inside human body.

The effect of pH on the growth of bacteria isolates was shown on figure 1. It was observed that all the bacterial isolates grew over a range of pH (5.0 –9.0). This was in

accordance with suggestion of Corlett and Brown (1980) that microorganisms could grow over a wide range of pH. The pH that supported the optimum growth of isolates *B. licheniformis* (BI_6) and *S.aureus* (BI_4) was 8.0 while *L.mesenteroides* (BI_1), *P.aeruginosa* (BI_3), and *B.cereus* (BI_5) grew best at 7.0. These results agree with the report of Griffin (1994) that pH and temperature are the two most important environmental factors that control the growth of microorganisms.

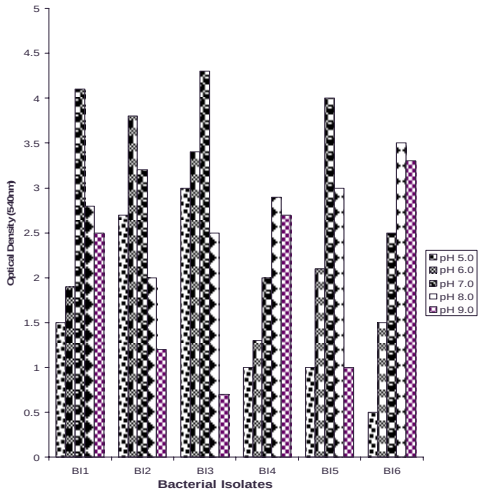

Fig. 1: Effect of pH on the Growth of Different Bacterial Isolates

In Nigeria, the major obstacle of artificial mushroom production is contamination from unwanted microorganisms. This normally leads to discouragement and economic loss to mushroom growers. A mushroom farmer must be able to identify and eradicate these microbial contaminants which could affect mushroom yield. This could be achieved by proper sterilization and incorporation of appropriate antibacterial agents into the medium used for mycelial propagation of these mushrooms. An ideal sterility should also sbe maintained during the spawning processes.

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