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Prebiotic potential and characterization of microbes found in the guts of Wistar rats fed with feeds supplemented with some Macro-fungi from Oyo state, Southwestern Nigeria

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Abstract

The new phenomenon in food technology is the use of prebiotics for therapeutic purposes to control the normal body flora of gastrointestinal tracts. The prebiotic potential and characterization of microbes found in the guts of Wistar rats fed with feeds supplemented with some selected macro-fungi diet was evaluated on male Wistar rats (Rattus norvegicus). Our result showed that the macro-fungi supplemented diets exhibited prebiotic activities while thebacteria isolated and characterized from the stomach of Wistar rat include Lactobacillus casei, Pediococusacidilactici, Lactobacillus fermentum, Enterococcus faecalis, Streptococcus pneumonia, Lactobacillus acidophilus, Leuconostoc lactis, Campylobacter jejuni, Vibroalbensis, Salmonella enterica, Shigella dysenteraie, and Escherichia coli. From the results, it is suggestive that the higher fungi enriched feedsis prebiotic as they enhanced the pro-biotic micro-flora over the pathogenic microbes.

Keywords: Prebiotic potential, Probiotic, Micro-flora and Macro-fungi.

Introduction

Mushroom's therapeutic qualities have been verified by extensive studies carried out around the world. Several scientists researched and examined the nutritional and therapeutic properties of mushroom growing worldwide[1]. The indigestible polysaccharides, which are the important source of prebiotics in mushrooms, help in the prevention of pathogens multiplication by escalating the proliferation of beneficial microbes in the gut[2]. Another way to strengthen the intestinal immune cells is to use certain growth promoters, such as pytobiotics, probiotics, and prebiotics as additives in foods[3]. They have beneficial effects on animal well-being, mainly due to improving the hosts' mucosal immunity through boosted immunity of the host mucosa and increased resistance to pathogenic bacterial colonization. The gastrointestinal tract of humans is a difficult environment where changes within the community are affected by the nutrients and the different species of micro-organisms available[4]. Some scientific shreds of evidence have indicated the impacts of gut microflora on host immune-modulatory activity and metabolic pathways that have helped inhibit a widespread of diseases. The majority of this impact is mediated mostly through diet and healthy foods taken by the host, thereby justifying the importance of diet modification that can alter the gut microflora positively[5].

Prebiotic is a fermentable nutrient, which can promote the growth of good bacteria in the colon of the host[6]. Healthy alteration of the host colon's composition and metabolic activity is of great importance due to the essential role of intestinal microflora in human health[7], in the production of vitamins and in improving the functions of the immune system[8]. Prebiotics are only necessary if they can move through the intestine to the colon, fermented by beneficial bacteria, and specifically support the growth of helpful intestinal bacteria[9]. The prebiotic activity score is being used to assess prebiotics' effectiveness in promoting probiotic or pathogen development.

In recent times, prebiotic consumption has been on the rise to increase probiotic production, with an increased quest for new prebiotic candidates[10]. The use of oral health prebiotics greatly increased the proportion of beneficial species, while reducing the percentage of harmful organisms[11]. This, however, has given a lead on the search for alternative sources, through probiotics and prebiotic feed additives. This research would, therefore, like to explore the potential use of macrofungi as a prebiotic and the characterization of the microflora found in the gut of Wistar rat.

Materials and methods

Mushroom sample collection and identification: During the rainy season (August-September 2016 and 2017) four separate fresh fruiting sections of the wild *Tramates versicolor*, *Lycoperdon rimulatum*, *Daedalea quercina* and *Ganoderma lucidum* were collected from Ibadan (7.3775°N, 3.9470°E)

Iseyin (7.9765^{o}N, 3.5914^{o}E), Ogbomosho (8.1227^{o} N, 4.2436^{o}E) and Saki (8.6726^{o} N, 3.3943^{o} E) respectively in Oyo State, Nigeria, and transported in a clean bag to the Botany Department Laboratory, University of Ibadan, and validated using standard definitions from[12] molecular classification method.

Figure-1: *Tramates versicolor* collected from Ibadan.

Figure- 2: *Lycoperdon rimulatum* collected from Iseyin.

Figure-3: *Ganoderma lucidum* collected from Saki.

Figure-4: *Daedalea quercina* collected from Ogbomoso.

Feed Formulations: Feed Formulations are mention in Table-1,2,3.

Table-1: Composition of Basal feeds for Rodent in 10 Kg.[13].

Class of Food	Weight (Kg)	% Composition
Maize	5.4	54
GNC	2.4	24
Soya beans	0.8	8
Wheat	1.0	10
Fish	0.4	4
Vitamins; A. Premix B. Lysin C. Metione D. Salt	0.0002kg 0.00014 kg 0.00014 kg 0.0002 kg	

Table-2: Composition of 20% higher fungi supplemented diet for Rodent in 10 Kg.

Class of Food	Weight (Kg)	% Composition
Maize	4.32	43.2
GNC	1.92	19.2
Higher Fungi	2.00	20.0
Wheat Soya beans Fish	0.80 0.64 0.32	8.0 6.4 3.2
Vitamins; A. Premix B. Lysin C. Metione D. Salt	0.0002kg 0.00014 kg 0.00014 kg 0.0002 kg	

Table-3: Composition of 40% Higher Fungi supplemented diet for Rodent in 10 Kg.

Class of Food	Weight (Kg)	% Composition
Maize	3.24	32.4
GNC	1.44	14.4
Higher Fungi	4.0	40.0
Wheat Soya beans Fish	0.6 0.48 0.24	6.0 4.8 2.4
Vitamins:A. Premix B. Lysin C. Metione D Salt	0.0002kg 0.00014kg 0.00014kg 0.0002kg	

Experimental design: For this study, a total of 154 healthy male Albino rats (Rattus norvegicus) were used with an average weight of 100g. They were brought from the Department of Physiology, Central Animal House, College of Medicine, Ibadan University, Nigeria. They were acclimatized for two weeks and held for two weeks before the experiment in solid bottom polypropylene cages under normal ambient room temperature conditions (23^{o}C-25^{o}C) and natural ambient 12-hour light-dark period, with free access to their basal diet feeds and water *ad libitum.* The animals were randomly divided into 22 groups, with each group consisting of seven rats: Each treatment group was duplicated, one for seven days, while the other group was split into 14 days. The rats were divided into 11 treatment groups (n=7): Group 1-(un-ulcerated normal feed (CN)) and ulcerated groups 2-11, Groups 2-(not treated (CU)), 3-(20mg/kg of cimetidine (Ccm)), 4-(20% of *Gl*), 5-(40% of *Gl*), 6-(20% of *Dq*), 7-(40% of *Dq*), 8-(20% of *Tv*) 9-(40% of *Tv*), 10-(20% of *Lr*) and 11-(40% of *Lr*). During the experimental studies, all the research animals were provided human care in compliance with the ethics and procedure outlined in the National Academy of Science (NAS) Handbook for the Treatment and Use of Laboratory Animals. Albus, U.[14] approved by the Institutional Animal Ethical Research Committee (UI-ACUREC/19/0039). Animals had unlimited access to water ad libitum during the cause of the tests.

Determination of the microbial counts: Faecal samples from the stomach of rats used for this experiment were collected and put inside test tubes containing buffer solution until when required[8] viable plate count method was used.

Characterization of the bacteria: Bacterial isolates were subjected to molecular analysis for identification as defined by Murray, M.G. et al[12].

Determination of prebiotic activities: The prebiotic activity was assessed by the method defined by Huebner, J.[15] based on the following equation:

Prebiotic activity score = [(PBP24H – PBP0H)/ (PBCN24H-PBCN0H)] - [(EPP24H- EPP0H)/EPCN24H- EPCN0H)]
PBP24H=Log CFU/mL probiotic growing on the prebiotic at 24hr.
PBP0H=Log CFU/mL probiotic growing on the prebiotic at 0hr.
PBCN24H=Log CFU/mL probiotic growing on the normal control (CN) at 24hr,
PBCN0H=Log CFU/mL probiotic growing on the normal control (CN) at 24hr,
EPP24H=Log CFU/mL enterics growing on the prebiotic at 24hr.
EPP0H=Log CFU/mL enterics growing on the prebiotic at 0hr.
EPCN24H=Log CFU/mL enterics growing on normal control (CN) at 24hr.
EPCN0H=Log CFU/mL enterics growing on normal control (CN) at 0hr.

Results and discussion

Figure-5 shows the effect of higher fungi supplemented diets on the microbial count in the stomach of the rat using MacConkey agar in rats for 7 and 14 day exposure periods. At day 7 treatment, no significant decrease ($^{j}p<0.05$) in microbial counts among all the treatment groups was observed while 40Dq, and 40Gl showed a significant decrease ($^{k}p<0.05$) in microbial counts when compared with the normal control (CN) treatment on day 14. At day 7 and 14 treatment, a significant increase in the microbial count with all the treatment groups when compared with normal control (CN) was observed as shown in Figure-6.

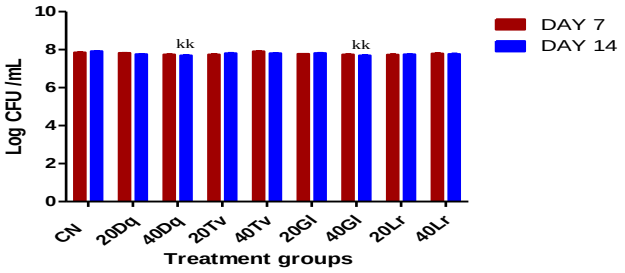

Figure-5: Effects of higher fungi supplemented diets on the microbial count in the stomach of the rat using Mcconkey agar for 7 and 14 day exposure periods.

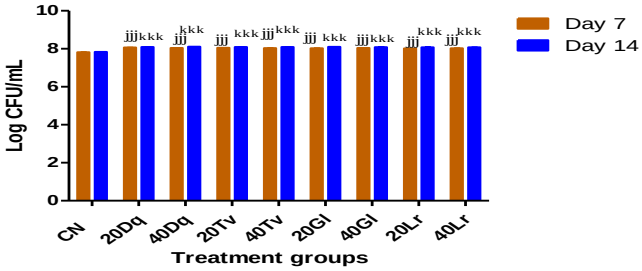

Figure-6: Effects of higher fungi supplemented diets on the microbial count in the stomach of the rat using MRS agar for 7 and 14 day exposure periods.

Keys of significance: Following one–way Anova, $^{j}p<0.05$, $^{jj}p<0.01$, $^{jjj}p<0.001$ at 7 days and $^{k}p<0.05$, $^{kk}p<0.01$, $^{kkk}p<0.001$ at 14days compared with the corresponding controls. Using two-way Anova, $^{z}p<0.05$, $^{zz}p<0.01$, $^{zzz}p<0.001$, between 7 and 14 days exposure periods.
20Dq = 20% of *Daedeliaquercina* in feed,
40Dq = 40% of *Daedeliaquercina* in feed
20Tv = 20% of *Tramates versicolar* in feed,
40Tv = 40% of *Tramates versicolar* in feed
20Gl = 20% of *Ganoderma lucidum* in feed,
40Gl = 40% of *Ganoderma lucidum* in feed
20Lr = 20% of *Lycoperdon rimulatum* in feed,
40Lr = 40% of *Lycoperdon rimulatum* in feed
The prebiotic activity score of the feeds used to ascertain the prebiotic activities of the feeds on Wistar rats studied is presented on Table-4. On day 7, the highest prebiotic activity score was observed with 20*Tv* at 0.044 and the least with 40*Tv* at 0.021 while on day 14, the highest prebiotic activity score was seen with 40*Dq* at 0.071 and the least with 40*Tv* at 0.036. The prebiotic activity scores increased with feeding days.

Twelve bacteria species were isolated from stomach samples of rats fed with higher fungi supplemented feeds used in this study. They were coded isolate Bac 1-12 (Table-5). These microorganisms were characterized as *Lactobacillus casei, Pediococcus acidilactici, Lactobacillus fermentum, Enterococus faecalis, Streptococus pneumonia, Lactobacilus acidophilus, Leuconostoc lactis, Campylobacter jejuni, Vibroalbensis, Salmonella enterica, Shigella dysenteraie, and Escherichia coli.*

Table-4: Prebiotic effects of higher fungi supplemented diets treatment on Wistar rat.

Treatment groups	Day 7			Day 14		
	Log Cfu/mL (MRS Agar)	Log Cfu/mL (Mcconkey Agar)	Prebiotic activity score	Log Cfu/mL (MRS Agar)	Log Cfu/mL (Mcconkey Agar)	Prebiotic activity score
CN	7.820	7.854	0.00	7.84	7.791	0.00
20*Dq*	8.072	7.826	0.035	8.10	7.657	0.05
40*Dq*	8.049	7.744	0.043	8.11	7.507	0.071
20*Tv*	8.053	7.747	0.044	8.10	7.651	0.051
40*Tv*	8.037	7.910	0.021	8.09	7.762	0.036
20*Gl*	8.029	7.777	0.037	8.10	7.668	0.049
40*Gl*	8.045	7.744	0.043	8.09	7.521	0.067
20*Lr*	8.021	7.741	0.040	8.08	7.711	0.041
40*Lr*	8.029	7.794	0.034	8.08	7.599	0.055

Table-5: Molecular identification of the microbes isolated from the stomach Sample of Wistar rat after treatment with higher fungi supplemented diet.

ID(NCBI) SUBMISION	Accession number	Identified name	Blast search similarity
Bac 1	FBUI-9	*Lactobaciluscasei*	99.95%
Bac 2	FBUI-11	*Pediococus acidilactici*	99.95%
Bac 3	FBUI-8	*Lactobacilus fermentum*	99.95%
Bac 4	FBUI-3	*Enterococus faecalis*	99.95%
Bac 5	FBUI-7	*Streptococus pneumoniae*	99.92%
Bac 6	FBUI-10	*Lactobacilus acidophilus*	99.92%
Bac 7	FBUI-12	*Leuconostoc lactis*	99.92%
Bac 8	FBUI-1	*Campylobacter jejuni*	99.83%
Bac 9	FBUI-4	*Vibroalbensis*	99.94%
Bac 10	FBUI-5	*Salmonella enterica*	99.98%
Bac 11	FBUI-2	*Eschericia coli*	99.99%
Bac 12	FBUI-6	*Shigella dysenteriae*	99.98%

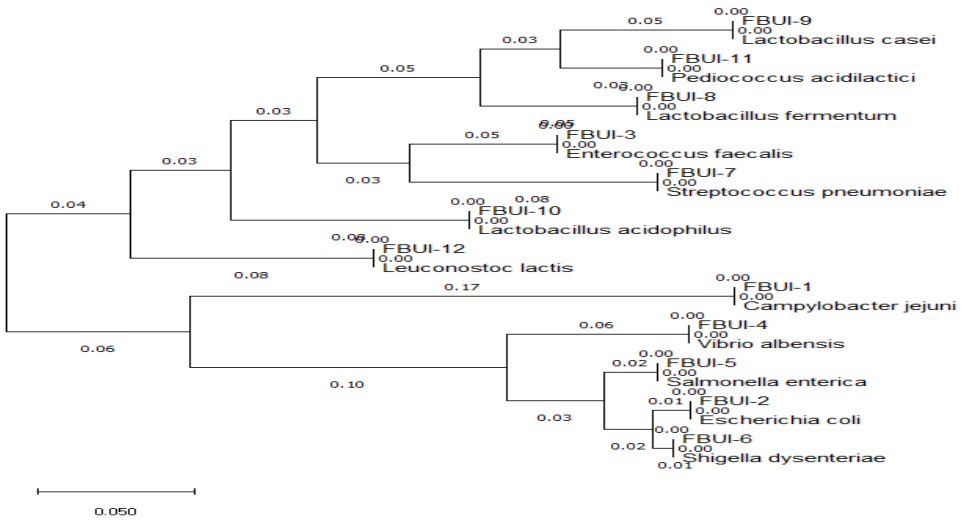

Figure-7: Phylogenetic relationship between the Bacteria.

Discussion: The use of prebiotics for medicinal purposes to regulate the natural body flora of the gastrointestinal tract is a recent phenomenon in food technology. Many scientists have researched the world of intestinal microbes for years now to count, classify, and describe them. However, the important role of gut microorganisms in food digestion, the defense of the gastrointestinal tract from the invasion of pathogens, and the production of essential vitamins are the result of these wide-ranging efforts. The host and the microbial community have a symbiotic relationship.

In this study, the prebiotic activities were carried out *in-vivo* using the microbial count of microorganisms found in the stomach of Wistar rats using MacConkey agar and MRS agar media. This increase of Lactic Acid Bacteria (LAB) in the stomach with the treatments could be linked with the physiological properties of LAB, such as anaerobic, and acidophilus, which make them survive the acidic state of the stomach caused by the fermentative role microorganisms play to break down the indigestible polysaccharides in the treatment feeds thereby enhancing their growth in the stomach. This is supported by the findings of Kumar, (201)[2], who reported that indigestible polysaccharides are essential sources of prebiotics in mushrooms which helps to prevent the replication of pathogens by increasing the proliferation of beneficial microbes in the intestines. It could be suggested that the higher fungi enriched feeds may be prebiotic as they are enhanced the pro-biotic microflora. This is further supported by the prebiotic score results observed in Table-4. This is in accord with the findings of Roberfroid*et al.*[4] that prebiotics are food substrates with health benefits that preferentially stimulate the growth of helpful colon microbes and prevent pathogenic species from proliferating.

Fifteen bacteria species coded Bac 1-15 were isolated from faecal samples of rats fed with higher fungi supplemented feeds used in this study. These microorganisms were characterized as *Lactobaciluscasei, Pediococusacidilactici, Lactobacilus fermentum, Enterococus faecalis, Streptococus pneumonia, Lactobacilus acidophilus, Leuconostoc lactis, Campylobacter jejuni,,Vibroalbensis, Salmonella enterica, Shigella dysenteraie, and Eschericia coli* (Table-6).This is in agreement with the findings of Zimmermann and Curtis[16] who reported that intestinal microbiome is composed of helpful and harmful bacteria, actively fighting for nutrients and space. The pure cultures of these lactic acid bacteria isolated could be incorporated into feeds as probiotics to enhance good health.

Conclusion

From the results, it is suggestive that the higher fungi enriched feeds are prebiotic as they enhanced the pro-biotic micro-flora over the pathogenic microbes.

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