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ANTAGONISTIC IMPACT OF ISOLATED LACTIC ACID BACTERIA FROM FISH GUT ON MYCOFLORA OBTAINED FROM FISH FEEDS

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Abstract

Biological decontamination using microorganisms has revealed new opportunities, currently only selected biological methods are acceptable for the decontamination of feed. This research work discusses the literature on fish feed, their composition, contamination of fish feeds by mycoflora or mycotoxic fungi, use of probiotics to remove or inhibit mycoflora and prevent their possible mechanisms of action. This study is aimed at selecting the best lactic acid bacteria isolate that can antagonize the fungi isolate from fish feed sample. The 10g of fish feed pellets sampled from 10 local and 10 foreign fish feeds were ground into fine particles using a blender, and then 10g each of the samples was added into 90mls of water and mixed thoroughly, using serial dilution of 10^1 to 10^4 by pour plate method, the last two diluent 1ml was added into the sterilised petri dish and a sterilized prepared Potato dextrose agar (PDA) media was added to the plate containing 1 ml of sample swirled briefly for even distribution and allowed to set before incubating the plates at 28^0C for 3-5 days. After 5 days visible growth were recorded and also microscopic identification was also conducted, picking the organism on a grease free slide and staining the isolate with lactophenol blue using cover slip to cover it and viewed with x40 magnification under the microscope. Every observations were recorded. Performing the antagonistic test 21 labelled unknown lactobacillus isolate sub cultured before were used to antagonize the fungi isolates culturing on the same plate using agar well plate method. The organisms were incubated at 28^0C for 3-5 days and growth of the two organisms were observed, with zone of inhibition measured between the two organisms and recorded. Using 9mm as bench mark for zone of inhibition It was observed that LAB isolates 24, 48, 114, 122 were the best LAB isolate that had inhibited more fungi isolate than the rest of other LAB Isolates based on the criteria that they were the highest that inhibited more zones and were able to inhibit almost all if not all fungi isolates with the most common toxin called aflatoxin secreted by *Aspergillus flavus*. LAB 24 is identified as *lactobacillus terrae* strain, LAB 48 is *lactobacillus plantarum* strain, LAB 114 is shown to be *lactobacillus brevis* and LAB 122 shows *pediococcus pentosaceus*. This research work shows that lactic acid bacteria can be used as probiotics to reduce the infection of fungi in aquatic animals especially fish. Finally attention should be paid to probiotics as it is best way on controlling or reducing the level of infection and mortality of fish in commercial fish farming and also preventing food poisoning to humans.

Keywords: Fish, feed, bacteria, Probiotics, Mycology

INTRODUCTION

The main features of fish feed are protein and fat content. The protein in the diet is mainly used for the accumulation of tissues, fat is the main source of energy and the increase of adipose tissue. The amount of carbohydrates in

fish feed is usually low because fish, especially carnivorous fish, are less able to digest carbohydrates. Therefore, the energy in the diet must come from fats and fats have higher energy density than carbohydrates. As a result, fish feed is more concentrated than that of farm animals on land, so protein levels (up to about 40-45%) and energy density are higher. The digestible protein/digestible energy ratio is an important feature of a fish feed and, as a rule of thumb, should more or less resemble the protein/energy ratio of the growing fish itself. In this way, maximum retention of dietary protein (an expensive component of fish feed) is achieved. The basic Ingredients For Fish Feed Formulation are fish meal, fish oil, rice bran, shrimp bran, wheat bran, soybean cake, cottonseed cake, dried potatoes etc (WHO, 2018). Fish feeds is contaminated as a result of mycotoxin contamination of crops which might occur in the pre-harvest stage, especially in the agricultural commodities which are bran or fiber rich and also have high mold and high moisture content. Contamination can also occur at the postharvest stage or during storage in inappropriate or unconducive conditions which will favor mycotoxin production that is when temperature and water activity rises to the level which will allow optimal conditions for fungal growth and mycotoxin production. Once an ingredient or finished feeds is contaminated there are currently no methodologies to eliminate the mycotoxins. Although different processing methods might help in reducing mycotoxin concentration especially those which uses higher temperatures; Mycotoxins endanger human and animal health, hinder international trade, causes food and feed waste, and consume plenty of resources from conducting research, enforcing regulation and finding solution to reduce the problem they cause. Moreover in the fish farming industry they cause economic losses as a result of poor fish growth, morbidity and mortality of aquatic organisms and increase in feed consumption. Fungi belonging to *Aspergillus, Alternaria, Claviceps, Fusarium, Penicillium,* genera, primarily produce mycotoxin. They widely contaminate food and feed supplies in the field or during storage. *Aspergillus* and *penicillium* species known as storage fungi, commonly grow on foods and feeds under storage conditions, however *fusarium* species often contaminate crops in the field and spread in the plant during growth (WHO, 2018). Probiotics are live microorganisms which when administered in the right amount confer health benefit to the host. They commonly belong to the genera *Lactobacillus* and *Bifidobacterium*. And can be found naturally in the gastrointestinal system of the host in both human and animals like mammals and most especially aquatic animals like fish. Probiotic organisms are also called beneficial microorganism. While mycotoxins are secondary toxic fungal products with a long history of responsibility for food borne disease outbreaks. Human and animals are continuously exposed to variable levels of these contaminants such as aflatoxins, ochratoxin A, fumonisins, deoxynivalenol, patulin, zearalenone, among others) that occur naturally in the diet. The long term exposure might cause tissue and genetic damage.

Certain probiotic strains can bind and remove mycotoxins from liquid media. Eukaryotic cell cultures showed that the complex probiotic-mycotoxin is less adhesive to enterocytes than the probiotic alone, then favoring maybe the elimination of this complex from the gut through feces. Probiotics were also shown capable of restoring some functions of the epithelial cells after the damage produced by mycotoxin exposure. Animal trials revealed that genetic damage and tissue oxidation might be also partially avoided by the oral administration of probiotics. Finally, experimental trials conducted in fish feeds naturally exposed to mycotoxins in feed that received probiotics, showed reduced levels of mycotoxin-DNA adducts in fish feeds and in the content of mycotoxins in fish (Vinderola and Ritieni, 2015). A study of Ochratoxin A degradation compared 27 commercial LAB strains cultivated for 24h at 37°C in 10 mL MRS broth contaminated with 0.6 mg/mL Ochratoxin A. The authors concluded that among the six strains that showed 97-99% total reduction of OTA at pH 6.5, hydrolysis was by far the predominant mechanism; only 2-4% were due to adsorption. Curiously, the hydrolysis was less effective in a more acidic medium (pH 3.5). Degradation products ochratoxin A and phenylalanine were confirmed by mass spectrometry. However, no protease was identified or even proposed, much less isolated and characterised. Interestingly, a study of Ochratoxin A reduction by *Lactobacillus.bulgaricus*, also tested the ability of these Lactobacilli to neutralize several

different trichothecenes such as nivalenol (1 ppm), deoxynivalenol (1 ppm), diacetoxyscirpenol (500 ppb) and T2 toxin (500 ppb), but no effect was observed. The same lack of correlation between detoxifying capacities was demonstrated for Ochratoxin A and patulin by *Lactobacillus* and *Bifidobacterium*. On the whole, the efficiency of LAB as Ochratoxin A scavengers is considerable and reinforces their role as probiotics with anti-mycotoxin action. However, as in the case of AFB1 detoxification, strains must be selected with great care as regards their capacity to neutralize Ochratoxin A and their optimal conditions. Post-harvest methods to prevent the growth of toxicogenic fungi include storage of crops under conditions of appropriate humidity and temperature, or the use of chemical fungicides. If, despite these methods, the products are contaminated with mycotoxins, treatments may be applied to reduce levels of mycotoxins. These include traditional and innovative physical methods (e.g., sorting, thermal treatment, UV radiation, cold plasma, electron beam irradiation, pulsed electric field, adsorbents), as well as chemical methods (addition of oxidants such as hydrogen peroxide, sulfur dioxide, sodium hypochlorite, ozone, or ammonia) (Marshal *et al*., 2020). However, some of these methods are not applicable in practice, mainly due to the risk of creating toxic residues or affecting the nutritional value and organoleptic properties of the purified products. Moreover, there are currently no legal regulations regarding decontamination of food. According to Regulation 1881/2006, foodstuffs that do not comply with accepted maximum levels of toxins should not be used as food ingredients, nor mixed with other foodstuffs, and should not be deliberately detoxified using chemical treatments. The presence of contaminants in food must be reduced as much as possible by Good Manufacturing Practice (GMP), Good Agriculture Practices (GAP), and the application of Hazard Analysis and Critical Points (HACCP). Sorting or other physical treatment methods make it possible to reduce the AF content in groundnuts, nuts, dried fruit, and cereals (El-Nezami *et.,al*, 1998). The regulations do not mention biological methods of decontamination.

Many of the fish farmed around the world are carnivores like the Atlantic salmon, Sea bass, and turbot. In the development of modern aquaculture, fish meals and fish oils were key component of feeds. They are mixed together with other ingredient like cereal grains, vitamins, minerals, and vegetable proteins formed into feed pellets. Wheats is widely used to bind the ingredients in the pellets. The feed contains a whole cell of Schizochytrium sp and rich protein defatted biomass of Nannochloropsis oculata as they were observed to work better on specific growth rate, growth, weight gain as they are called the best feed conversion ratio and fish nutrient content compared to the diet of ocean derived fish meal and fish oil (Sarker *et al*., 2020). Contamination of fish feeds comes as a result of mycotoxin contamination of crops which might occur in the pre-harvest stage, especially in the agricultural commodities which are bran or fiber rich and also have high mold and high moisture content. Contamination can also occur at the postharvest stage or during storage in inappropriate or unconducive conditions which will favor mycotoxin production that is when temperature and water activity rises to the level which will allow optimal conditions for fungal growth and mycotoxin production (Saad, 2016). Concerning sterigmatocystin, it has been found to contaminate agricultural commodities used as feed ingredients such as maize, soybean or barley, corn, wheat (Viegas *et al*., 2018). The contamination of fish feeds with mycotoxins or mycoflora are common and a worldwide problem, but the prevalence and type of depend mainly on the geographical location. For example Aflatoxin are mostly found in Africa, Southern Europe, Southeast Asia, and South Asia, Zearelenone contamination is found in North and Southeast Asia, Central Europe, North and South America, Africa, Ochratoxin A was prevalent in Africa and South Asia , deoxynivalenol is present in Northern and Central Europe, Africa and North Asia, and North America, and fumonisin has higher prevalence in Southern Europe, Southern America,

North, South, and Southeast Asia, and Africa (Pinotti *et al*., 2016).

In Nile tilapia intoxification of ochratoxin resulted to sluggish swimming, decreased growth performance, reduced survivability, refusal to eat, reduction in total protein, and degenerative lesions in the liver and kidney with necrosis, among other effect (Diab *et al*., 2018). In humans Ochratoxin A has a genotoxic effect which result in DNA damage which is the first step to carcinogenesis. Exposure to this toxin may result in the development urinary tract tumors, testicular cancer, hepatic cancer (Malir *et al*., 2016). Temperature or climate change could cause crops to be susceptible to mycotoxins production as a result of fungal growth thereby causing damage to the crops before, during and after harvesting which in turn is a serious threat to food safety and security (Magan and Medina, 2018). Therefore the trade export of commodities or food of the country will be at a reduced value; making the price of the item to be low which is a big disadvantage to the country's economy thereby creating room for poverty (Gbashi *et al*., 2017).

Probiotic organisms are live microorganism that provides health benefit when consumed, by improving or restoring the gut microbiota (NHS, 2018). Probiotics are considered safe for consumption but may cause bacteria-host interactions and unwanted side effects in rare cases (Doron and Snydman, 2015). Though they are numerous benefits claimed and marketed towards using consumers probiotic products such as improving immune Health (Turck *et al*., 2019), reducing gastrointestinal discomfort, avoiding the common cold, and relieving constipation. Probiotics serve as an alternative antimicrobial agent to reduce the dependence on vaccine, antibiotics, and other drugs, and also to improve the fish health in aquacukture and obtain safe fish product of high quality as found in many various studies (Dawood and Koshio, 2016). The use of probiotics as a protective agent prevents the spread of diseases and improves the composition of microbiota (Nandi *et al*., 2018). Probiotic organism enhance innate immunity and resistance to disease reducing stress resulting from sharp change in the composition of the diet, technological stress and other causes and violation of feeding regimes (Soltani *et al*., 2017).

MATERIALS AND METHODS

Materials

The materials used included glass slides, conical flasks, petri dishes, beakers, durham tubes, McCartney bottle, weighing balance, incubator, spirit lamp, glass covered jars, autoclave, inoculating loop, cotton wool, microscope, hot air oven, glass slide and cover slip, blender, some quantity of fish feeds.

Sterilisation of Materials

The laboratory glass wares such as McCartney bottles, conical flasks, pipettes, beakers were sterilized using an autoclave at a temperature 121^0C and a pressure of $1kg/cm^2$ for 15 minutes. Materials such as inoculating loops were sterilized by heating red hot in a flame. All media unless otherwise stated, were sterilized by autoclaving.

Collection of Samples

10 each of 2kg of imported fish feed and Another 10 sample of 2kg of Local fish feeds were obtained from a Livestock feeds plc market outlet at Agric bus stop, Ile-epo Ekoro way, Abule-Egba, Lagos, both local and foreign samples were obtained and was taken to the laboratory for analysis in the Federal Institute of Industrial Research, Oshodi, Lagos.

1 Moisture content of fish feed sample

A small dish container was weighed first and recorded, after that 5*g* of the fish feed sample was weighed into the dish container and recorded. Then the dish containing the fish feed sample was placed in the oven for 15-20 minutes to dry at 105^0C, after which they were brought out weighed and recorded. Then the moisture content was calculated.

Therefore the percentage moisture content is calculated as

$$\frac{Loss\ of\ weight}{weight\ of\ sample}\ x\ 100$$

$$\frac{W_2 - W_3}{W} x\ 100$$

key: W = weight of sample
W1 = weight of dish
W2 = weight of dish + sample
W3 = weight of dish + sample of constant weight

Media preparation

The laboratory medium used for the isolation of fungi microorganisms from the fish feed was Potato dextrose agar (PDA). Another medium used for subculturing already prepared lactic acid bacteria was De Man, Rogosa and Sharpe agar (MRS).

lnoculation of samples

Isolation of fungi (mold) from fish feed samples

Each pellet was weighed 10*g* from 10 foreign samples of fish feeds and was placed in a mortar and using the pestle it was ground to a fine particle, the fine particle of foreign fish feeds, This was done for the 10 foreign samples. Another 10g pellet was weighed for 10 local samples and was ground using pestle and mortar thoroughly into fine particles and was done for 10 local samples. Another 90*ml* of distilled water was prepared in test tubes and was then autoclaved at 121^0C for 15 minutes.

Another 39*g* of potato dextrose agar (PDA) and 52*g* of malt extract agar (MEA) were weighed using a digital chemical balance and suspended into 1 litre amount of distilled water each, homogenized on hot plate magnetic stirrer to form a uniform solution.

10*g* of ground fish feeds where added into 90*mls* of distilled water and were mixed thoroughly, from the solution 1*ml* was added to test tube containing 9*mls* of distilled water Made up of 10*ml* of diluent dispersed into test tubes for serial dilution of samples. The media and diluent were sterilized at 121^0C for 15 minutes in an autoclave. At the end of the period the media were cooled to 45^0C in the water bath in order to inhibit bacterial growth, streptomycin were aseptically weighed and added to the two media separately. Using serial dilution and pour plate method/technique, 1*ml* of the ground fish feeds was added to 9*ml* of the sterilized distilled water in each of the test tubes and then mixed thoroughly using serial dilution from 10^1 to 10^4. After which 1*ml* of the last two dilutions was then plated in petri dish with a selective medium used. The cool molten potato dextrose agar (PDA) and the malt extract agar was poured in the petri dish contained of 1ml of the blended sample of fish feeds in each of the 20 plates (a duplicate of the 10 plates), the plates were swirl clock wisely and anti-clock wisely for evenly distribution of the inoculum. The agar was then left for some time to set and each plate was labelled from F_1 to F_{10} and also L_1 to L_{10} before placing it into the incubator at 28^0C for 5 days. At the end of incubation period, the colony growth observed on the culture plates were counted. The colony of the visible count per ml was calculated by multiplying the average number of colony per countable plate by the reciprocal of the dilution and reported as colony forming units/mL (cfu/mL)

Sub culturing of the obtained colonies

Various colony growth obtained from the inoculated petri dishes were sub cultured by picking the organism and dropping on sterile Potato dextrose agar plates and incubated for 5 days at 28^0C. The re-inoculation process was repeated until pure culture were obtained, These were stored in a refrigerator until when needed.

Identification of fungi and lactic acid bacteria isolates

Cultural appearance

Standard parameters such as color of the colony, shape of the colony, elevation, and texture of color surface, size, structure of fruiting organ and spores color, shape and size aerial mycelial growth were used to describe the colonies of the obtained fungi isolates.

Wet staining

The instrument used in identifying the isolate is light microscopy, after incubation of the inoculated samples for 3-5 days the growth of the fungi isolate becomes visible. Each of the labelled plates F_1 to F_{10} and L_1 to L_{10} was then brought out of the incubator and then using inoculating loop the fungi were picked from each of the labelled and placed on the clean grease free glass slide on which a drop of distilled water had been dropped, these was done for each of the 20 plates after which each of the slides containing the fungi organism was

stained with the lactophenol cotton blue stain and covered with a coverslip. Each of the slides was then placed under a microscope using both x10 and x40 magnification. The observation of each of the labelled organism was recorded according to the view under microscope.

Gram's staining

Identification was done using the Gram staining method on 62 isolates of already sub cultured lactic acid bacteria. Standard Gram staining procedures as described by (Fuchs *et.al.,* 2008) were carried out on each of the isolates obtained. A drop of normal saline was dropped in a clean grease-free slide and little quantity of the isolate was emulsified on the drop of saline. The smear was air-dried and heat fixed by passing it over flame. Some drops of crystal violet were added to smear and allowed for 30-60 seconds, then washed off, by rinsing under running tap water. The slide was then flooded with Gram's iodine, to add the stain, rinsed off after 30-60 seconds, and later drained. The slides were flooded with 95% ethanol to decolorize the smear, washed off immediately with water and drained. Safranin red was added as counter stain. It was left to stand for 2 minutes and washed off. The smear was drained, air dried, and a drop of immersion oil added. The color, shape of the
cells in the smear were observed using light microscope with immersion lens.

RESULTS AND DISCUSSION

Moisture content of the fish feed sample

The moisture content of the result below indicates the moisture of both the local and imported fish feed samples which is interpreted be that 11.16% to 11.28% is said to be the range in percentage moisture content in local sample while 9.22% to 9.34% is the percentage range in moisture content for the imported feed samples which is still tolerable as a good criteria for fish feeds (Table 1).The moisture content of fish feed sample from imported and local feed which range from 9.22-11.28% in percentage moisture falls into the normal moisture content as according to (Md *et. al*.,2016) which highlighted that moisture content of cereal based feed should not be above 12-13%, and deterioration of feed can be as high as 20-30% (Md *et al*., 2016). Therefore the moisture content of the samples is within the optimal moisture content for fish feeds.

Fungal colony count obtained from both local and imported fish feed and the morphology

Looking at the table below, colony count that is highest is found in the imported sample with isolate code F_9 which gives us 1.3×10^5, therefore the number of fungal colony is more in imported samples than the local sample due to the exposures of the sample to inappropriate storage conditions, and they are usually stored for a lengthy period of time before being sold and the possible growth of fungus present there is the *Aspergillus flavus* which is a dangerous fungi that produces aflatoxin unlike the local sample which has the lowest colony count which is 1.0×10^4, from $L_{3,4,5,6,8,10}$. They have a lesser colonial growth because they are not stored for too long the likely growth present there is *Fusarium solani and Aspergillus niger* they also produce toxins dangerous to fish which are ochratoxin A, Fumonisin, zeralenone and trichothecenes. Humidity and temperature levels during the storage of feed ingredients and compounded feeds strongly influence the formation of mycotoxins. Besides potential impairment of the feed quality, e.g., due to decreasing vitamin levels and changes in the nutritional composition of the feeds (Alabi *et al.,* 2017), bioactive and toxic compounds including mycotoxins can be formed during feed storage.

Antagonistic test of lactic acid bacteria against fungal isolate

Using 10mm as the bench mark for zone of inhibition, the result shows LAB isolate 16 was used to antagonize isolate of mold from sample F1 to F10 and the result was that four (4) samples were seen to exhibit zone of inhibition from imported fish feeds mostly *Aspergillus flavus*, While from the local sample five fungi isolates were inhibited mostly *Aspergillus niger*. LAB 22 isolate was able to inhibit 4 fungi isolates each from both imported and local samples which mostly are *Aspergillus flavus* and *Aspergillus niger*

Table 1: Moisture content of the fish feed sample for both Imported and local samples

Sample name	Weight of sample (W)	Weight of dish (W1)	Weight of dish + sample (W2)	Weight of dish + sample of constant weight (W3)	Moisture content (%)
Local sample	5.0g	46.561g	51.561g	50.997g	11.28%
	5.0g	45.925g	50.925g	50.367g	11.16%
Imported sample	5.0g	44.845g	49.845g	49.378g	9.34%
	5.0g	47.265g	52.263g	51.802g	9.22%

KEY: W = weight of sample
W1 = weight of dish
W2 = weight of dish + sample
W3 = weight of dish + sample of constant weight

Table 2: Number of fungal colonies obtained from both imported and local fish feed samples and their morphological appearance

Fungi isolate code	Number of colonies (cfu/g)	Morphological Appearance
F_1	8.0×10^4	Greenish dark round colony with feathery spot
F_2	8.0×10^4	Small white colony
F_3	1.1×10^5	Green and brown spot in the middle and whitish brown on exterior
F_4	7.0×10^4	Green colonies with brown yellow spots on organism and white colony spot on the exterior of organism
F_5	1.0×10^5	Greenish colony and white colony with touch of yellow spot in the middle and white spot surrounding organism
F_6	9.0×10^4	Greenish colony with white spot on the middle and exterior of the colony
F_7	9.0×10^4	Dark concentrated spot numerous
F_8	$7{,}0 \times 10^4$	More of whitish colonies around the organism with faint greenish colonies
F_9	1.3×10^5	Greenish colony with white spot in the middle and white round the colony
F_{10}	7.0×10^4	Small round whitish concentrated colonies
L_1	2.0×10^4	White dispersed spots scattered round the medium
L_2	3.0×10^4	Whitish round spots
L_3	1.0×10^4	Dark brown concentrated small spots
L_4	1.0×10^4	Dark brown concentrated small spots
L_5	1.0×10^4	Dark numerous spots
L_6	1.0×10^4	Dark numerous concentrated spot
L_7	2.0×10^4	Dark concentrated numerous spot
L_8	1.0×10^4	Pink red colonies dispersed
L_9	2.0×10^4	Dark numerous concentrated spot
L_{10}	1.0×10^4	Dark spot concentrated numerous

LAB 24 isolate was seen to inhibit 6 fungal isolates from imported samples and 6 isolates from local samples mostly both *Aspergillus flavus* and *Aspergillus niger*. LAB 28 isolate was seen to inhibit 6 fungal isolates which are mostly *Aspergillus fumigatus* and none in particular for the local sample. LAB 30 were seen to inhibit 5 fungi isolates which are mostly *Penicillium digitatum*, and for the local sample *Aspergillus niger* is inhibited mostly. LAB 42 was seen to inhibit 4 fungal isolates which are mostly *Aspergillus flavus* from imported

sample and *Aspergillus niger* from local sample. LAB 45 isolate were seen to inhibit 4 and 7 fungal isolates which were mostly *Aspergillus flavus* and *niger* from both imported and local sample respectively. LAB 46 isolates were also seen to inhibit 4 fungi isolates which are between *Aspergillus fumigatus*, and *Aspergillus flavus* and *Aspergillus niger* for local sample. LAB 47 were seen to inhibit 6 fungi isolates which are *Aspergillus fumigatus* in imported samples and 7 inhibited which were mostly *Aspergillus niger*. LAB 48 isolate were seen to inhibit 6 and 7 fungal isolates which are *Aspergillus flavus* and *Fusarium solani* respectively in both imported and local samples. LAB 49 isolate were seen to inhibit 5 fungi isolate each were isolated from imported and local feed sample mostly *Aspergillus flavus*, and *Aspergillus niger*. LAB 52 isolates were seen to inhibit 4 fungal isolates which are mostly *Penicillium digitatum* from imported samples and for the local sample 5 fungal isolates were inhibited which are mostly *Aspergillus niger*. LAB 73 were seen to inhibit 6 fungi isolate which were mostly *Aspergillus flavus* for the imported samples and 5 fungi isolates mostly *Aspergillus niger* for local samples. LAB 74 isolate were seen to inhibit 5 fungi isolates which were mostly *Aspergillus fumigatus* for imported samples while for local no specific fungi. LAB 85 were seen to inhibit 2 fungi isolates which are mostly *Aspergillus flavus* in imported sample while in local sample 3 fungi isolates were inhibited mostly *Aspergillus niger*. LAB 103 isolate were seen to inhibit 5 and 3 fungi isolates which are mostly *Aspergillus flavus* and *Aspergillus niger* from both imported and local samples. LAB 114 were seen to inhibit 6 and 5 fungi isolates respectively from both inported and local samples which are mostly *Aspergillus flavus* and *Aspergillus niger*. LAB 122 were seen to inhibit 8 fungi isolate which are *Aspergillus flavus* for imported samples and for local samples 3 isolates inhibited mostly *Fusarium solani*. LAB 123 isolates were seen to inhibit 7 fungal isolates which is between *Penicillium digitatun and Aspergillus flavus* in imported samples, as for the local sample mostly *Aspergillus niger* is inhibited. LAB 129 isolate were seen to inhibit 4 fungal isolates which were mostly *Aspergillus fumigatus* and for the local samples 4 fungi isolates were inhibited mostly *Fusarium solani*. And finally the LAB 135 isolate were seen to inhibit 2 fungi isolates which were mostly *Aspergillus flavus* from imported sample and 4 fungi isolate inhibited mostly *Aspergillus niger*.

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Table 3: Antagonistic test of lactic acid bacteria against fungi isolate from both foreign and local fish feed samples

LAB ISOLATE CODE	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	L1	L2	L3	L4	L5	L
16	9	1	0	35	5	10	10	2	10	5	3	1	5	14	11	6
22	10	5	0	10	11	8	25	4	5	5	1	5	10	5	5	12
24	50	3	7	5	15	20	7	10	15	1	8	1	9	17	4	35
28	25	5	10	10	22	7	13	1	7	10	10	50	10	25	7	5
30	10	10	9	5	7	25	16	5	5	10	50	0	1	7	5	10
42	7	8	8	15	5	14	7	10	7	11	20	5	0	19	10	3
45	20	0	7	7	15	15	5	5	14	5	10	1	20	10	15	25
46	55	2	10	5	15	7	5	1	10	5	5	1	7	14	5	15
47	60	5	10	8	6	10	20	15	1	25	7	0	0	15	20	17
48	5	0	11	20	20	8	24	10	4	20	10	0	4	20	13	25
49	5	40	5	30	17	20	10	5	5	7	60	5	5	12	10	20
52	10	10	2	30	5	8	2	5	5	10	8	5	10	5	10	15
73	7	0	45	5	16	5	15	10	10	14	9	1	0	5	6	10
74	10	10	15	5	7	4	5	20	20	5	5	0	9	32	14	8
85	8	5	6	25	8	10	4	5	8	7	8	2	7	17	1	5
103	10	4	5	20	10	10	13	7	3	4	4	7	5	5	5	5
114	5	40	10	15	14	16	4	9	14	9	12	1	10	7	10	1
122	5	1	10	15	10	25	15	15	10	13	7	0	13	10	7	8
123	7	63	10	45	5	15	10	15	5	10	10	45	6	25	9	20
129	13	0	9	20	7	8	15	1	8	15	10	60	10	10	2	5
135	8	1	1	1	10	2	6	5	15	8	6	0	15	7	1	10

Note: The measurement of each value above is in millimeter (mm)
KEY: 0 – 10 mm = Resistant F1 – *Aspergillus fumigatus* L1 – *Mucor piriformis*

10– 20 mm = Sensitive
20 mm above = Very sensitive.

F2 – *Penicillium digitatum* **L2 – *Penicillium digit***
F3 – *Aspergillus fumigatus* **L3 – *Fusarium solani***
F4 – *Aspergillus flavus* **L4 – *Fusarium solani***
F5 – *Aspergillus flavus* **L5 – *Aspergillus niger***
F6 – *Aspergillus flavus* **L6 – *Aspergillus niger***
F7 – *Aspergillus niger* **L7 – *Aspergillus niger***
F8 – *Rhizopus stolonifera* **L8 – *Fusarium avenaceum***
F9 – *Aspergillus flavus* **L9 – *Aspergillus niger***
F10 – *Penicillium digitatum* **L10 – *Aspergillus niger***

Antagonistic test of LAB isolates against fungi isolates

From the result above, it shows the antagonistic reaction from both the 21 lactic acid bacterial isolates and the fungal isolates in total between imported and local feeds, using the benchmark for zone of inhibition to be 10mm the LAB isolate $_{85}$ inhibited 5 fungi isolates out of 20 which is the lowest in total including imported and local feeds, followed by LAB $_{135}$ which inhibited 6 fungal isolates out 20, LAB $_{46}$ is the next which inhibited 7 fungal isolates, LAB $_{22}$, LAB $_{103}$, LAB $_{30,}$ LAB $_{74,}$ and LAB $_{129}$ inhibited 8 fungal isolates out of 20, LAB $_{16}$, and LAB $_{52}$ inhibited 9 fungi isolates in total between imported and local feed, LAB $_{42}$, and LAB $_{49}$ inhibited 10 fungi isolates out of 20 in total, followed by LAB $_{45}$, LAB $_{47}$,LAB $_{73,}$ LAB $_{114,}$LAB $_{122,}$ and LAB $_{123}$ Inhibited a total of 11 fungi isolates out of 20 between imported and local fish feeds, LAB $_{24}$, and LAB $_{28}$was able to inhibit a total of 12 fungi isolates out 20, and finally LAB $_{48}$was able to inhibit a total number of 13 fungi isolates which is the highest zone of inhibition out of the all the LAB isolates used to antagonize the fungi isolates which is in agreement with Teixeira *et al* (Teixeira *et al*., 2021). Based on the toxins produced by the fungi isolated F_4, F_5, F_6 and F_9 were identified to be *Aspergillus flavus* which produces Aflatoxin which is the most common and dangerous toxin out of the toxins produced by the fungi isolates, isolate LAB $_{114}$ and LAB $_{122}$ were able to inhibit all *Aspergillus flavus* from the imported feed sample, while LAB $_{48}$ isolates was able to inhibit 3 out 4 *Aspergillus flavus* but were the highest overall in terms of zone of inhibition in both the imported and local inhibition samples, then isolates 16 and 24 which also was able to inhibit 3 out of 4 fungi isolates but LAB $_{24}$ had an edge over Lab $_{16}$ because LAB $_{24}$ is among the highest number of isolate overall .

Therefore the most antagonistic LAB isolates are LAB $_{24}$, LAB $_{48}$, LAB $_{114}$ and LAB $_{122}$ due to the highest number of fungi isolates inhibited and also they were able to inhibit 12,13,11 fungal isolates respectively out of 20 fungi isolates. Also these listed isolates were picked on the bases of the number of *Aspergillus flavus* inhibited by the LAB isolates and the concentration or number of colonies. Although *Aspergillus niger* was mostly identified in the local sample there concentration or number of colonies were not much which is equally insufficient to cause potential harm or danger to aquatic animals and eventually humans.

CONCLUSION

In conclusion, this research work establishes that fish feeds can be contaminated with toxic fungi, The work also shows that lactic acid bacteria can be used as probiotics to reduce the chances of fungal infection in fish and eventually humans as they help to inhibit the growth of fungi that produce the toxins that cause the infection. Therefore more attention needs to be paid in using lactic acid bacteria as probiotics in the treatment of fish infections especially in commercial fish farming as it will help to reduce the risk of increasingly cases of food related diseases in Nigeria.

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