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Full Length Research Paper

Antimicrobial potentials of indigenous *Lactobacillus* strains on gram-negative indicator bacterial species from *Clarias gariepinus* (Burchell.) microbial inhibition of fish-borne pathogens

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This study tries to evaluate the ability of *L. fermentum* LbFF4 isolated from Nigerian fermented food (*fufu*) and *L. plantarum* LbOG1 from a beverage (*ogi*), to inhibit some fish bacterial pathogens (*Citrobacter*, *Enterobacter*, *Escherichia coli*, *Klebsiella*, *Proteus*, *Pseudomonas* and *Salmonella* species) under *in vitro* conditions. Overall phenotypic antibiotic resistance of 124 Gram-negative bacterial isolates obtained from the gut contents of cat fish, *Clarias gariepinus*, using agar disc-diffusion method, indicated very high resistance to amoxicillin (88.8%), augmentin (85.1%), cotrimoxazole (80.6%), tetracycline (72.3%), gentamicin (61.9%) and nalidixic acid (57.5%). Multiple antibiotic resistance (MAR) of 37.5 - 87.5% were also recorded. None of the forty four *Lactobacillus* strains (*L. brevis*, *L. delbruekii*, *L. fermentum*, *L. plantarum* and *L. reuterii*) isolated from the gut contents of *C. gariepinus* was inhibitory *in vitro* towards the Gram-negative bacterial species. However, *L. fermentum* LbFF4 and *L. plantarum* LbOG1 exhibited *in vitro* antibacterial activities against 41.1 and 47.6% of the Gram- negative bacterial species respectively. The susceptibility patterns of the indicator pathogens using the probiotic candidates were significant. The potential probiotic candidates were able to survive relatively low pH (3.5 - 5.5) and the fish bile. This study therefore, signifies that lactobacilli-based probiotic candidates from Nigerian fermented foods and beverages can serve as adjunct, inhibitory food-supplements in the control of bacterial fish-borne pathogens of *C. gariepinus*.

Key words: Antibiotic resistance, antimicrobials, aquaculture, *Clarias gariepinus*, *Lactobacillus*, probiotics.

INTRODUCTION

The African catfish, *Clarias gariepinus* (Burchell.) (Huisman and Richter, 1987) was one of the most suitable species for aquaculture in Africa (Hogendoorn, 1979) , and since the 1970's, has been considered to hold great promise for fish farming especially Nigeria.

However, there is the food safety concern about consumption of the fish, such as in the case of food poisoning. One of the major problems in aquaculture is fish diseases (Austin and Allen-Austin, 1985; Toranzo et al., 2005; Li et al., 2009) and serious infections have been reported in those countries in which aquaculture are reasonably developed, including Nigeria, South Africa, Zambia and Zimbabwe. Numerous diseases have emerged as serious economic or ecological problems in aquaculture species, meanwhile, very little work has been done on fish diseases in Nigerian aquaculture, and as earlier suggested in the past situation needs to be addressed as a matter of urgency because it is better to have prior information upon which proactive control measures can be implemented, rather than to have reactive research into problems that have manifested themselves. It is also necessary to build research and diagnostic capacities in the sub-Saharan African region to deal with fish disease problems (Hecht, 2000; Jamu and Brummett, 2002), since the rate and extent of emergence

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can be reduced by the application of biosecurity programmes designed to mitigate the risk factors for disease emergence (Murray and Peeler, 2005; Boyd, 2009; Mikkelsen et al., 2009).

Fish and fishery products are in the forefront of food safety and quality improvement because they are among the most internationally traded food commodities. In 2001, fish trade amounted to US$ 54 000 million, of which approximately 50% originated from developing countries (Huss, 1994). Food quality, including safety, is however, a major concern facing the food industry today and a number of surveys have shown that consumer awareness about the quality of their food is increasing. The use of antimicrobial agents in aquaculture and the ultimate development of antibiotic resistance among bacterial flora from fish have been identified (Midvedt and Lingass, 1992; Aoki, 1997). The feed of the livestock, most importantly chicken and pigs, is supplemented with various antimicrobials for growth promoting, prophylactic and therapeutic treatments, meanwhile, such animal manures are excreted into fish ponds in most cases and antimicrobials and antimicrobial residues through the manure can enter the pond and establish a selective pressure in favour of antimicrobial-resistant bacteria (Petersen and Dalsgaard, 2003).

The transfer of resistance to commonly used antibiotics between microorganisms also adds to the consumers' fear and unease about what they eat. Therefore, with increasing demand for environment friendly aquaculture, the use of probiotics in aquaculture is now widely accepted (Vine et al., 2004; Balcázar et al., 2006; Wang et al., 2008; Qi et al., 2009). Many recent papers have deepened the state of knowledge about lactic acid bacteria (LAB) in fish gut (Jankauskiene, 2002; Balcázar et al., 2008), and in spite of high variability in fish microbiota, LAB are sometimes abundant in the intestine, notably in freshwater fish. Most LAB are harmless, while some strains have been reported for beneficial effects on fish health. Antagonism to pathogens is also another main feature of probiotic candidates (Gatesoupe, 2008).

Since the effects of probiotics have been found to be dependent on factors such as geographical locations and diets (Murray, 1990; Franz et al., 1999; Qi et al., 2009), the aim of the present pilot research study therefore, is to determine the possibility of developing indigenous, antimicrobial-producing bacteria of food origin that are in the class considered as generally regarded as safe (GRAS) in aquaculture practices, as food-based, microbial supplements in the control of fish-borne pathogens in Nigerian farmed cat fish (*C. gariepinus*).

MATERIALS AND METHODS

Collection of samples

Intact gut contents of *C. gariepinus* samples were separately collected in ice packs from six different locations (Agbowo, Agodi, Bodija, Eleyele, Samonda and Sango) within Ibadan metropolis, Oyo State Nigeria. The collected samples were labeled in batches according to the time and locations of collections and were processed for microbial analyses less than 2 hours after collection. Palm wine, *burukutu*, *ogi*, *garri* and *fufu* samples, which are some Nigerian fermented foods and beverages were purchased from various locations within Ibadan Abeokuta and Ijebu Ode metropolis and immediately transported to the laboratory for microbial analyses.

Microbiological analyses

The gut contents of the fish samples were separately dissected under aseptic conditions with sterile scalpels and forceps and scooped with sterile micro spatulas into two sets of sterile McCartney bottles containing sterile De Man, Rogosa and Sharpe (MRS) broth (pH 5.5) and peptone water (pH 8.5). The broth culture media containing the gut contents were incubated for 12 h at 30°C before subsequent serial dilutions of the samples in 9ml aliquots of sterile distilled water to dilution factor of $10^{-5.}$

Isolation and characterisation of Gram-negative bacterial isolates

The pour plating method was employed in the culturing of the samples on different sterile culture media (Lab M, Basingstoke, England) - cysteine lactose electrolyte deficient agar (CLED), thiosulphate citrate bile sucrose agar (TCBS), eosin methylene blue agar (EMB), *Salmonella-Shigella* agar (SSA) and plate count agar (PCA). The cultured agar plates were then incubated at 35^0C for 24 – 36 hrs before repeated sub-culturing to obtain pure cultures of the bacterial flora. Pure cultures of the Gram-negative bacterial strains were kept in triplicates on brain heart infusion agar slants. Phenotypic taxonomic studies were carried out on the pure Gram-negative bacterial isolates based on their cultural, morphological, biochemical and physiological characteristics (Paick, 1980; Holt et al., 1994; Crichton et al., 1996).

Isolation and characterisation of *Lactobacillus* strains

Ten ml of MRS broth (LAB M, Basingstoke, England) containing the gut contents of the catfish samples and the fermented starchy foods and beverages were aseptically transferred into sterile 10 ml MRS broth at pH 5.5 - 5.7 and incubated for 12-18 hrs at pH 5.5 - 5.7 and incubated overnight at 35°C. One milliliter aliquots of each incubated broth culture were separately transferred to sterile Petri dishes followed by the addition of sterile MRS agar before incubating anaerobically at 35°C for 24 h. Cream to white circular, low convex colonies which were glistening and entire edged were subcultured by streaking to obtain pure cultures. Pure cultures of the *Lactobacillus* strains were kept in triplicates on MRS agar slants as bench cultures, while the *Lactobacillus* stock cultures were stored in Hogness freezing medium (3.6 mM K_2HPO_4; 1.3 mM KH_2PO_4; 2.0 mM Na- citrate; 1.0 mM $MgSO_4$; 12% glycerol) and kept frozen until used.

Grams reaction and cell shape of the *Lactobacillus spp* were determined using 24 h old pure cultures grown in MRS broth. The isolates were examined microscopically and initial confirmation and grouping of the lactobacilli were based on Gram's and catalase reaction, growth at 15 and 45°C in MRS medium; growth in 4% mannose, xylose according to the method of de Mann, Rogosa and Sharpe, (1960). The isolates that met the result for the results for preliminary identification criteria were separately grown in replicates for (18 - 24 h) in 10 ml MRS broth at 35°C. The purity of the strains was checked and the bench isolates were kept on MRS agar slants in the refrigerator. Fermentation of various sugars by the tentatively

Table 1. Phenotypic antibiotic susceptibility profiles of the gram-negative bacterial species isolated from gut contents of *C. gariepinus*.

	% antibiotic susceptibility / zones of inhibition (mm diameter) discs (µg)								
	Bacterial species	**Amx**	**Cot**	**Nit**	**Gen**	**Nal**	**Ofl**	**Aug**	**Tet**	**MAR (%)**
1	*Citrobacter* [33]	0.0	0.0	51.5	36.4	39.4	75.8	18.2	18.2	37.5 – 87.5
		-	-	10.0-22.0	10.0-16.0	10.0-30.0	16.0-30.0	10.0-30.0	10.0-26.0	*1
2	*E. coli* [18]	5.6	16.7	61.1	22.2	22.2	77.8	0.0	5.6	50.0 – 87.5
		12.0	16.0-22.0	10.-24.0	10.0-18.0	12.0-20.0	15.0-28.0	-	12.0	*1
3	*Enterobacter* [9]	22.2	22.2	55.6	44.4	22.2	77.8	0.0	77.8	37.5 – 75.0
		12.0-18.0	16.0-22.0	10.0-21.0	10.0-18.0	18.0	12.0-30.0	-	18.0-22.0	*2
4	*Klebsiella* [25]	0.0	32.0	68.0	32.0	44.0	84.0	12.0	20.0	37.5 - 87.5
		-	10.0-20.0	10.0-20.0	11.0-18.0	10.0-24.0	12.0-30.0	12.0-28.0	10.0-25.0	*1
5	*Proteus* [12]	0.0	8.3	58.3	25.0	25.0	91.7	0.0	8.3	37.5 – 87.5
		-	16.0	10.0-26.0	10.0	10.0-24.0	12.0-30.0	-	12.0	*1
6	*Pseudomonas* [4]	50.0	25.0	50.0	50.0	75.0	75.0	25.0	25.0	37.5 – 87.5
		10.0-16.0	12.0	10.0-25.0	10.0-12.0	12.0-28.0	12.0-28.0	10.0-22.0	12.0	*2
7	*Salmonella* [23]	0.0	4.3	56.6	34.8	47.9	91.3	0.0	26.1	37.5 – 87.5
		-	20.0	10.0-22.0	10.0-15.0	10.0-28.0	12.0-30.0	-	10.0-20.0	*2
	Overall Suscep.	5	16	72	41	47	102	10	27	
	% Overall Suscep.	4.0	12.9	58.1	33.1	37.9	82.3	8.1	21.8	
	Total rest.	119	108	52	83	77	22	114	97	
	% Total rest.	96.0	87.1	41.9	66.9	62.1	17.7	91.9	78.2	

Key: Amx = amoxicillin; Cot = cotrimoxazole; Nit = nitrofurantoin; Gen = gentamicin; Nal = nalidixic acid; Ofl = ofloxacillin; Aug = augmentin; Tet = tetracycline . Values in parenthesis are the % resistance; * = no. of strains with 100.0% MAR.

identified *Lactobacillus* strains was further carried out. Phenotypic identification of the *Lactobacillus* strains was based on the cultural, microscopic, biochemical and physiological characteristics of the strains, while the confirmation of their identities was according to the methods of Kandler and Weiss (1986) Sneath et al. (1986) and Molin et al. (1993) (Table 1)

Antibiotic susceptibility determination of bacterial strains

Using the agar disc diffusion-method, the Gram-negative bacterial isolates from the gut contents of *Clarias* fish were screened against the most commonly used antibiotics in Nigeria [discs - amoxicillin (AMX 25 µg); augmentin (AUG 30 µg); cotrimoxazole (COT 25 µg); gentamicin (GEN 10 µg); nalidixic acid (NAL 30 µg); nitrofurantoin (NIT 200µg); ofloxacillin (OFX 30 µg); tetracycline (T 30 µg);] according to the NCCLS (2003) method.

Bioassay of antimicrobial activities of the lactobacilli strains:

The 24 - 36 h old lactobacilli broth (MRS) cultures were screened for their inhibitory activities against the indicator Gram-negative bacterial isolates from the gut contents of *Clarias* fish, using the modified agar well diffusion-method of Tagg et al. (1976). Seeded plates were prepared by transferring and streaking approximately 1.5 × 10^5 cfu/ml culture broth of each indicator bacterial strain on Mueller-Hinton agar (MHA) surface. The seeded agar plates were left for about 15 min before aseptically dispensing the 500 µl of each 24 - 36 h putative lactobacilli broth culture (already incorporated into sterile, semi-solid plain agar) into the agar wells already bored in the agar plates. The plates were then incubated at 32°C for 18 - 24 h. Zones of inhi - bition were measured and recorded in millimeter diameter.

RESULTS

In this study, a total of one hundred and twenty

Table 2. *In vitro* inhibition of Gram-negative indicator bacterial species from the gut contents of *C. gariepinus* by the antimicrobial-producing *Lactobacillus* strains.

	% bio-inhibition / zones of inhibition (mm diameter)	
Gram-negative bacteria	***L. plantarum* LbOG1**	***L. fermentum* LbFF4**
Citrobacter species [33]	45.5	51.5
	(10.0 - 18.0)	(12.0 - 21.0)
Enterobacter species [9]	55.6	44.4
	(12.0 - 18.0)	(10.0 -18.0)
Escherichia coli [18]	33.3	22.2
	(10.0 - 21.0)	(14.0 - 23.0)
Klebsiella species [25]	48.0	36.0
	(10.0 - 22.0)	(10.0 - 18.0)
Proteus species [12]	41.7	58.3
	(10.0 - 19.0)	(12.0 - 18.0)
Pseudomonas species [4]	50.0	25.0
	(10.0 - 16.0)	(12.0)
Salmonella species [23]	60.9	82.6
	(12.0 - 21.0)	(10.0 - 18.0)
% Total susceptibility	47.6	41.1

The values in parenthesis indicate the diameter of inhibition zones in mm using 500 l of the lactobacilli in MRS culture broth (agar well–diffusion) on Mueller Hinton agar. Clear zones and zones 10.0 mm in diameter were indicated as resistant (R); zones 10 were indicated inhibitory.

Four Gram-negative bacterial isolates obtained from the gut contents of cat fish, *C. gariepinus* (Burchell) were characterised as *Citrobacter* 33 (26.6%), *Enterobacter* 9 (7.3%), *E. coli* 18 (14.5%), *Klebsiella* 25 (20.1%), *Proteus* 12 (9.7%), *Pseudomonas* 4 (3.2%) and *Salmonella* 23 (18.5%) species.

The overall phenotypic antibiotic resistance profiles of the gram-negative bacterial species from the gut contents of the farmed *C. gariepinus* indicated that they were highly resistant to amoxicillin (96.0%), augmentin (91.9%), cotrimoxazole (87.1%), tetracycline (78.2%), gentamicin (66.9%) and nalidixic acid (62.1%) but moderately resistant to nitrofurantoin (41.9%). Theantibiotic resistance rates however, varied among the different species, *Citrobacter* (48.5 - 100.0%); *E. coli* (38.9 - 94.4%); *Enterobacter* (44.4 - 100.0%), *Klebsiella* (56.0 - 100.0%) except for tetracycline, in which 22.2% resistance was recorded among *Enterobacter* and nitrofurantoin, in which 32.0% resistance was recorded among *Klebsiella* species; *Proteus* (41.7 - 100.0%); *Pseudomonas* (50.0 - 75.0%) and *Salmonella* (52.1 - 100.0%). Overall lower resistance (8.32 - 25.0%) was recorded towards ofloxacillin. Multiple antibiotic resistance (MAR) of 37.5 - 87.5% was also recorded among the bacterial species, while at least, one of the bacterial species had 100.0% MAR (Table 2).

The 44 *Lactobacillus* strains isolated from the gut contents of *Clarias* in this study were identified as *L. brevis* 2 (4.5%); *L. delbruekii* 13 (29.5%); *L. fermentum* 18 (40.9%); *L. plantarum* 10 (22.8%) and *L. reuterii* 1 (2.3%), while those isolated from some Nigerian fermented foods and beverages were identified as *L. brevis* 4 (8.7%); *L. casei* 1 (2.2%); *L. delbruekii* 12 (26.1%); *L. fermentum* 19 (41.3%); *L plantarum* 9 (19.6%) and *L reuterii* 1 (2.2%).

None of the *Lactobacillus* strains from the gut contents of the *C. gariepinus* fish was inhibitory against the Gram-negative bacterial species but two of the *Lactobacillus* strains from two Nigerian fermented foods, *L. plantarum* LbOG1 from *ogi* and *L. fermentum* LbFF4 from *fufu* were inhibitory *in vitro* against 47.6% and 41.1% of the selected Gram-negative bacterial species respectively.

DISCUSSION

One of the most serious problems facing sub-Saharan aquaculture is the introduction of pathogens and other diseases by way of introducing alien species of fish or shellfish for farming purposes (Austin and Allen-Austin, 1985), which can reside in the environment or on / in apparently normal fish (latent carriers) (Wedemeyer, 1996; Olga and Haenen, 2001 Toranzo et al., 2005). The dominant and common Gram-negative intestinal bacterial flora of most fishes such as trout, salmonids, carp and

clariads that could be cultured in Petri plates include *Aeromonas, Alcaligenes, Carnobacterium*, *Citrobacter* and other Proteobacteria, *Acinetobacter*, *Enterobacter*, *E. coli*, *Klebsiella*, *Pseudomonas*, *Moraxella, Shewanella, Vibrio*, *Photobacterium* and *Flavobacterium* spp. (Austin and Gibb, 1993; Ayulo et al., 1994; Noga, 1996; Apun et al., 1999; Asai et al., 1999). In the previous study of Adewoye and Lateef (2004), the Gram-negative bacterial species isolated from the body surfaces of *C*. *gariepinus* in Nigeria were those in the genera, *Proteus, Pseudomonas, Serratia, Enterobacter* and *Escherichia*, while in this current study, similar genera of Gram-negative bacterial species were isolated from the gut contents of *C*. *gariepinus*.

Some fish pathogens remain infectious as suspended in the water column or attached to the sediment particles, and when shed into the water by an infected host, they usually remain viable long enough to be transmitted horizontally (fish to fish) (Ringø and Birkbeck, 1999). To support this assertion, Fujioka (2001) reported that *Escherichia coli* and *Salmonella* can survive for very long periods in tropical waters and once introduced may almost become indigenous to the environment. This is also possible in some other fish pathogens of foodborne importance. Therefore, there is the possibility that some bacterial species obtained from *Clarias* can serve as potential bacterial aetiological agents of fish-borne, environmental or human infectious diseases of aquatic origin. Consequently, the presence of such foodborne pathogens on fish or fish products is of clinical significance from the foodborne infections point of view (FDA, 1997; WHO, 1999).

Different methods are available for reducing the populations of fish pathogens in aquaculture. Though bacterial diseases on commercial fish farms are currently treated with antibiotics, this form of treatment has clinical implications, more so, as identified that there is emergence of antimicrobial resistance following the use of antimicrobial agents in aquaculture (Midtvedt and Lingaas, 1992; Smith et al., 1994; DePaola et al., 1995; Aoki, 1997; Alderman and Hastings, 1998; WHO, 1999; Akinbowale et al., 2006). Feeds that were not eaten may also be consumed by other fish in the vicinity of a fish farm (Bjorklund et al., 1990). It is also well established that antibiotics given to animals have resulted in the emergence of some resistant flora that can infect humans via the food chain. Potential risks to consumer health therefore, exist as well, in that antimicrobial resistance arising from the use of antibiotics in aquaculture can be transferred to human bacterial strains. Studies that examined antibiotic resistance following drug therapy at fish farms (Bjorklund et al., 1990; McPhearson et al., 1991; Spanggaard et al., 1993; Dixon, 1994; Cole et al., 2009) have shown an increased frequency of resistance to several drugs across a variety of bacterial species.

In this study, although the resistance patterns were not species specific, general high resistance to the test antibiotics, especially amoxicillin (88.8%), augmentin (85.1%), cotrimoxazole (80.6%), tetracycline (72.3%), gentamicin (61.9%) and nalidixic acid (57.5%), as well as moderate resistance to nitrofurantoin (38.8%) were observed among the Gram -negative bacterial isolates obtained from *C* . *gariepinus*. Similar high antibiotic resistance had been previously observed among the bacterial species isolated from the body surfaces of *C. gariepinus* in the study of Mc Phearson et al. (1991); Adewoye and Lateef (2004), in which the resistance of the bacterial were also isolates to the commonly used antibiotics in Nigeria indicated 100% (augmentin, amoxicillin and cloxacillin); 85.71% (tetracycline), 80.95% (cotrimoxazole) and 71.43% (erythromycin) rates. Multiple antibiotic resistance (MAR) of 37.5 - 87.5% was also recorded among the bacterial species in this study, while at least, one of the bacterial species had 100% MAR.

As earlier noted (Stewart and Sinigalliano, 1990; Spanggaard et al., 1993; Kruse and Sorum, 1994), antibiotic resistance patterns of the strains can lead to acquired, relatively higher resistance and transfer of antibiotic resistance genes to other normal flora of the fish that are not fish pathogens, as well as fish consumers and fish farmers / workers (Weber et al., 1994; Minchin, 2007), hence, conferring serious health problems. There is need therefore, to control such aquaculture problems (Wyatt et al., 1979; Noga, 1996). A growing concern for the high consumption of antibiotics in aquaculture has therefore, initiated a search for alternative methods of fish disease control (Gildberg et al., 1997). Chemical therapy of fish is also expensive; therefore, there is a need to investigate alternative treatment technologies and more environmentally acceptable forms of fish diseases treatment. In spite of great efforts in research, foodborne diseases continue to present a major problem of both health and economic significance. The cost of food borne disease is high and although the full economic impact is not fully known, preliminary estimates in the United States in 1994 placed the cost between US$ 10 - 83 billion (FDA, 1997). It has therefore, become overwhelmingly clear that all countries need an adequate food control programme to ensure a safe food supply to protect and promote the health of the consumers.

Many African foods are fermented before consumption and *Lactobacillus* have been found to be of great significance in some of such food fermentations. The reasons for their wide spread use in the preparation of food and other fermentation processes include their ability to discourage spoilage and contamination by other microorganisms, improve the nutritional value of food, as well as possession of therapeutic value and inhibitory effects on food borne pathogens (Svanberg et al., 1992; Brink ten et al., 1994; Ogunshe et al., 2007), However, a unique characteristic is that the inhibitory activities of lactic acid bacteria have been found to be species-specific and dependent on factors such as diets (Duncan et al., 2003; Hopkins and Macfarlane, 2003; Ogunshe, 2006). Several strains of *Streptococcus* are known to be pathogenic to fish (Gatesoupe, 2008), therefore, in trying

to select potential indigenous antimicrobial-producing microbial flora for aquaculture among the lactic acid bacteria, only *Lactobacillus* species were assayed for in this study.

None of the Gram-negative bacterial strains from *C. gariepinus* was inhibited *in vitro* by the lactic acid bacteria which were also isolated from the gut contents of the fish. In a similar study by Gildberg et al. (1997), no obvious growth inhibition of a virulent strain of *Vibrio anguillarum* by the test lactic acid bacteria, *Carnobacterium divergens* was observed in an *in vitro* mixed culture of *V. anguillarum* and *C. divergens* isolated from cod intestines. Two *Lactobacillus* strains that were isolated from two Nigerian fermented foods, *ogi* and *fufu*, *L. plantarum* LbOG1 and *L. fermentum* LbFF4 inhibited over 40% of the Gram-negative bacterial strains from the *Clarias* fish *in vitro*. While the conventional antibiotics (discs), with the exception of loxacillin were only able to inhibit between 11.2 - 42.5% of the Gram-negative bacterial strains from the *Clarias* fish *in vitro*, the *Lactobacillus* strains were able to inhibit 41.1 - 47.6% of fish-borne bacteria. In addition to the fact that antibiotics and chemical preservatives are no more permitted in foods as food additives in most countries, and where permitted the number of chemical preservatives approved for use in food is actually quite small the statistical significance of the inhibition results obtained in this study indicated the potential of the *Lactobacillus* as bio-antimicrobial.

Since there are considerable interests in ways of stopping the upward trend and reducing the incidence of food poisoning, antibiotic resistance in food-borne bacteria and also due to negative consumer perceptions of artificial preservatives, attention is shifting towards alternatives that the consumers perceive as natural, such as probiotics (Smith- Palmer et al., 1998; Vine et al., 2004; Balcázar et al., 2006, 2007, 2008; Ogunshe, 2008; Wang et al., 2008; Qi et al., 2009). A major step forward in recent years was the converging evidence that LAB can stimulate the immune system in fish (Gatesoupe, 2008) , this research study has therefore, tried to identify some of the prominent and easily recoverable gram-negative bacterial pathogens in African catfish (*C. gariepinus*) in Nigeria, as well as making efforts to inhibit the growth of such bacterial species *in vitro* using microbial metabolites of potential probiotic *Lactobacillus* species from Nigerian indigenous fermented foods, thereby serving as alternative chemotherapeutic agents in fish farming practices. Further studies on characterisation of the antimicrobial metabolites and survival of the *Lactobacillus* strains during transit in the gut of African catfish (*C. gariepinus*) are on-going in our laboratories.

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