Original Research Article

Probiotic Bacteria in Gastro-Intestinal Tract of Hybrid Catfish fed Insect Protein meal

Abstract

The evaluations of insect protein as viable alternative protein sources in the diet of fish are gaining more attention revealing that insect protein has more comparative advantage than other protein sources. Therefore, this study was conducted to isolate and identify bacteria species using phylotypic and genotypic characterization in the gastrointestinal tract (GIT) of hybrid catfish fed supplemented grasshopper meal. Four feeding diets were used (Diet A (conventional feed), Diet B (100% *Grasshopper meal),* Diet C (50% fishmeal and 50% Grasshopper meal*)* and Diet D (100% Fish meal). At the end of 12weeks feeding period, the fish were sacrificed for their GIT to determine bacteria diversity. In diet A, the bacteria isolates identified were *Streptococcus pyogenes, Enterobacter aerogene, Staphylococcus aureus, Shigella flexneri, Escherichia coli, Leuconostoc latis* and *Klebsella pneumonia.* Whilst, *Bacillus subtilis, Lactococcus bulgaricus, Lysinibacillus macroides, Lysinibacillus capsici, Stenotrophomonas pavanii* and *Lysinibacillus* sp were found in GIT of fish fed diet B. Similarly, the fish fed diet C recorded the presence of *Bacillus subtilis, Lactococcus bulgaricus, Lysinibacillus* sp., *Staphylococcus aureus, Lysinibacillus macroides, Stenotrophomonas pavanii* and *Enterobacter aerogene.* The bacteria isolate in the GIT of fish fed diet D were *Bacillus cereus, Staphylococcus aureus, Pedicoccus acidilactis, Escherichia coli, Salmonella typhi, Leuconostoc latis* and *Shigella flexneri.* Probiotic bacteria were more in fish fed with Diet B and C which is an indication that the different ratio of supplemented grasshopper meal is good for the fish, as they may boost the fish immune system and improve the microbial balance of the gastro-intestinal tract.

Keywords; Bacteria, Freshwater fish, Grasshopper, Nutrition.

Introduction

The report of FAO, (2015) has put forward that fish farming is rapidly becoming the bailout point of the protein need of Africans. In Nigeria fish serves as the highest contributor of animal protein and accounts for over 34% of all the animal protein utilized in the country (Shinkafi and Ukwaja, 2010). In Africa, Nigeria ranks top in terms of usage of aquaculture feed in both local and international sources as result of its fish production level (Akinrotimi *et al*., 2011). Fish contributes immensely to worlds protein as it improves nutrition with high biological value, high protein retention, presence of amino acids which is essential with low level of cholesterol (Musefiu, 2016). The cost of fish production has increased astronomically due to the cost of fish which is a major protein source, thereby making fish culture business more tedious with lesser to profit or sometimes losses. In order, to reduce the pressure on the use of fish as protein source in their diet; studies have been advocating for supplementary feed with insect protein having more comparative advantage (Adamu *et al*., 2021a, Adamu *et al*., 2021b; Takakuwa *et al*., 2022, Adamu *et al.*, 2022).

Amongst the commonest edible insect in Nigeria is the grasshoppers (*Gomeophocerippus rufus*) that are rich in amino acid profile just like the fishmeal (Olaleye, 2015). Many researches have advanced that grasshopper meal can used in the composition of animal and fish feed (Olaleye, 2015, Adamu *et al.*, 2022). Similarly, research has shown that grasshoppers are high in protein, vital amino acids and presence other nutrients (Ganguly *et al*., 2014). Several studies have demonstrated that fishmeal dietary protein might be partly substituted in fish diets with various grasshopper meal without impacting growth, feed utilization efficiency and reproduction (Ganguly *et al*., 2014; Ghosh and Mandal, 2019). Furthermore, this insect's dry matter includes around 64% crude protein, a significant quantity of important amino acids, lipids, minerals, vitamins and a low amount of anti-nutritional elements (Ghosh *et al*., 2016; Ghosh and Mandal, 2019).

The gastrointestinal tract of an animal is made up of diverse and dynamic microbial ecology (Lio-po *et al*., 2002) which is vital in terms of nutrition, physiological function and pathology (He *et al.*, 2016). Fish digestive are rich in nutrients and provides a favorable growing environment for bacteria in compared to the surrounding water (Nayak, 2010). The presence of bacteria in fish could play diverse roles; some might be beneficial (probiotics) as they will improve the immunity response and mechanism of physiology in the fishes (Al-harbi

and Uddin, 2003). However, some bacterial species could lead to post harvest spoilage and adverse health conditions (Amande and Nwaka 2013). Mondal (2008) reported lack of information on gut micro flora in fish as these microbes serve as an important path in the fermentation of unused energy substrates, averting growth of harmful specie and control the production of vitamins for the host (Guarner *et al*., 2005).

Probiotics have been employed as a disease control method in aquaculture activities particularly those caused by infection of bacteria (Caipang *et al.,* 2020; Hasan and Banerjee 2020; Yang *et al*., 2022; Hortillosa *et al*., 2022). Lactic acid bacteria which originate in the GIT of fish are form of probiotic bacteria that are of benefit to fish health through greater nutrient utilization and antimicrobial capabilities (Hasan and Banerjee 2020). Probiotics boost the immune response to pathogenic illnesses in fish (Agustina *et al*., 2022; Hortillosa *et al*., 2022; Yang *et al*., 2022). Various studies have demonstrated that specific probiotic groups have antibacterial action in vitro on rainbow trout (Balcázar *et al*., 2008), common carp (Kaktcham *et al*., 2017), Tilapia (Zapata and Lara-Flores 2013) and other species of freshwater fishes (Alonso *et al*., 2019; Hanol *et al*., 2020). *Lactococcus lactis, Enterococcus* spp., *Lactobacillus plantarum* and *Leuconostoc mesenteroides* are examples of bacteria that produced lactic acid which have probiotic potential in fishes (Ringo *et al.,* 2018; Adamu *et al.,* 2022).

Recently the used of molecular and genetic tools as means of species identification have gained attention worldwide and has resulted in the used of 16S RNA in identification to species level. Many microorganisms' diversity, distribution, and behavior have been studied via phylogenetic identification (Emelianoff *et al.,* 2008). Many research has employed phylogenetic trees to investigate the evolutionary history and the relatedness of bacteria populations (Bonifassi *et al*., 1999). Previously, phenotypic traits were utilized in bacterial identification; however, most phylogenetic studies are now based on DNA sequences and this molecular information may be presented as a phylogenetic tree (Bonifassi *et al*., 1999); thus, the techniques to be adopted in this study. Thus, the study was aimed at isolating and identifying bacteria isolates using both phylotypic and genotypic methods in gastrointestinal track of hybrid catfish fed with supplemented grasshopper meal 12 weeks' trial period.

Materials and Methods

Study area

The research was carried out in the Aquaculture section of the Biological Garden, Department of Biological Science, Ibrahim Badamasi Babangida University, Lapai, Niger State., Nigeria, which lies between latitude of 9º0674ºN and longitude 6º5698ºE.

Preparation and formulation of experimental diet

The diet materials were crushed individually into the same sizes using a grinding machine and then weighed according to the quantities required for each diet. The amount of each component was measured using a sensitive weighted balance. Following thorough and accurate mixing of the components, starch was added to the feed to function as a binding factor in holding the feed together. Hot water was added to create a dough-like mixture that could be smooth pelleted using a pelleting machine. The pellets were sundried to reduce moisture before being wrapped in airtight polythene for storage in a dry environment.

Preparation of the feeds was through modified Pearson's square method (Adamu and Nwadukwe, 2013) with all diet having equal amount of brown seaweed (*Sargassum muticum*) as shown in table 1 (Adamu *et al.,* 2021a) and varying amount of Grasshopper meal. Four feeding diets were used thus, Diet A (Blue crown feed) manufactured by crown flour limited, Diet B (100% Grasshopper meal), Diet C (50% fishmeal and 50% Grasshopper meal*)* and Diet D (100% Fish meal).

Table 1: Feed ingredients composition of the practical diets.

Ingredients	Diet A	Diet B	Diet C	Diet D
Fishmeal	(2mm of blue crown feed)	-	13.76	22.71
Grasshopper meal	-	31.64	13.76	-
Sargassum	-	3.00	3.00	3.00
Maize flour	-	53.36	57.48	62.29
Vitalyte	-	2.00	2.00	2.00
Baobab	-	5.00	5.00	5.0
Cassava flour	-	5.00	5.00	5.00
Total	100	100	100	100

Fish Collection

A total of eight hundred fingerlings of hybrid catfish with and average live weight of 15g were purchased from a reputable fish farm (Alhassan fish farm in Abuja). The fishes were transported to biological garden of Ibrahim Badamasi Babangida University in a 50-litre filled plastic container. Acclimation was allowed for two weeks, during which they were fed

with commercial fish feed pellets, before gradually introducing them to the formulated feed as they were fed twice daily (Morning and evening). After acclimation, the fish were randomly distributed to the experimental concrete tanks in two replicate, each with thirty-five (35) fingerlings in semi-static bioassay system. The tanks were drained bi-weekly to prevent accumulation of organic matter, as the fish were weighed to measure the growth parameters.

Experimental set-up

The experimental set-up consists of twelve (12) outdoor concrete tanks with a capacity of 1.3 m × 0.9 m × 1 m (1.17 m^3). Each tank was assigned to each experimental diet containing different meal levels. The water level was remained at 0.8 m throughout the experimental phase with replenishment at two days interval.

Sample Collection for bacteria isolation

After the 12week feeding trial, twenty fish per diet were selected and sacrificed. Their Gastro-Intestinal tract were carefully removed and placed in sterile glassware. Each specimen was dissected aseptically to remove the gut contents for analysis.

Bacteria isolation and identification

Bacteria identification was characterized by phenotypic method in accordance with the description of Cheesebrough (2006). biochemical test (Gram staining test, indole test, catalase test, coagulase test, oxidase test, sugar fermentation test, citrate test and urease test) whilst, some of the isolates identified by phylotypic method were further subjected to genotypic method of identification using 16S rRNA technique and sequences compared in Gene bank.

The culture media (Nutrient agar, EMB agar, MRS agar, and MacConkey agar) were prepared and dispersed on a sterile Petri-dish according to the manufacturer's instructions. The Petri dishes were inverted to prevent condensation from droppings from the plate's cover onto the media's surface. The media was allowed to gel by chilling before being correctly labeled (Cintron, 2002).

Sterilized swab stick was rolled over the inoculation areas (fundus, duodenum, small intestine and large intestine (colon)) to maximize transfer of organism. For 24 hours, Petri plates were incubated at 37°C (Cheesebrough, 2006). All isolates were sub-cultured and transferred to slant medium to create a pure culture before performing various biochemical assays (Cheesebrough, 2006).

Molecular characterization of bacteria isolate

After the series of biochemical tests for the isolate's identification, some isolates were subjected to further genotypic identification that involves DNA extraction, PCR (Polymerase chain reaction), Gel electrophoresis, Gel extraction and Sequencing using 16S rRNA.

Bacteria isolate was cultivated overnight in an Eppendorf tube, spun down at 14,000rpm for 2 minutes, supernatant was removed, and DNA was extracted using the CTAB technique (Thottappilly *et al.*, 1997; Akinyemi and Oyelakin, 2014). After that, the DNA was resuspended in 100µl of sterile distilled water. The DNA concentrations in the samples were assessed, as well as the genomic purity. The DNA was then checked on a 1.0% agarose gel and visualized using a UV light source. The MJ Research Thermal Cycler was used for the PCR analysis (PTC-200 model). The primer utilized for PCR amplification was a 16S universal primer for bacteria, with the forward primer sequence being 5'AGAGTTTGATCCTGGCTCAG3' and the reverse primer sequence being 5'ACGGCTACCTTGTTACGACTT3'. The PCR mixture contains 1l of 10X buffer, 0.4l of 50mM MgCl2, 0.5l of 2.5mMdNTPs, and 0.5µl of 5mM 0.5µl of 5µmM forward primer The PCR profile utilized contains an initial denaturation temperature of 94°C for 3mins, followed by 30 cycles of 94°C for 60 seconds, 56°C for 60 seconds, 72°C for 120 seconds, and the final extension temperature of 72oC for 5 minutes and the 10°C hold forever. Before sequencing, the amplicons were further purified using Sodium Acetate washing methods. The pellet was resuspended in 5µl of sterile distilled water. The PCR mix used was 0.5µl of BigDye Terminator Mix, 1µl of 5X sequencing buffer, 1µl of 16S Forward primer with 6.5µl of distilled water, and 1µl of the PCR product, for a total of 10µl. The Sequencing PCR profile is a Rapid profile, with an initial Rapid thermal ramp to 96°C for 1 minute, followed by 25 cycles of Rapid thermal ramp to 96°C for 10 seconds, Rapid thermal ramp to 50°C for 5 seconds, and Rapid thermal ramp to 60°C for 4 minutes, followed by Rapid thermal ramp to 4°C and hold forever. Prior to sequencing, the PCR sequence product was purified using 2M Sodium Acetate washing techniques.

The pellet was resuspended in 5µliters of sterile distilled water. A mixture of 9µl of Hi Di Formamide and 1µl of Purified Sequence was made and placed into an Applied Biosystem (Hutter *et al.*, 2005; Matsheka *et al.*, 2005; Akinyemi and Oyelakin, 2014; Akinyemi *et al.*, 2016)

The resulting sequences were compared to the NCBI gene database using the BLAST search tool (www.ncbi.nlm.nih.gov). The proportion of sequence matching was also examined. The

BLAST homology search yields varying percentages of maximal identity for all isolate sequences. The bacterial genomes were aligned using the BioEdit program, and the aligned sequences were saved in a FASTA format, which was then uploaded to the MEGA 11 software for phylogenic analysis.

Phylogenetic analysis

The evolutionary history was constructed using Saitou and Nei's (1987) Neighbor-Joining technique. The best tree is given, and the evolutionary distances were determined using the Maximum Composite Likelihood technique and are measured in base substitutions per site (Tamura *et al*., 2004). This research comprises of seven nucleotide sequences. The following codon locations were included: 1^{st} + 2^{nd} + 3^{rd} + non-coding. For various sequence pair all unclear locations were deleted (pair-wise deletion option). The final sequence dataset had 485 locations in total. The evolutionary analysis was perform using the MEGA11 software (Tamura *et al*., 2021)

Results

Phenotypic identification

Bacteria isolated based on different diets (A-D) is shown in Table 2 below. Fishes fed with diet B, C and D were fed with different level of supplemented Grasshopper, Fish meal and brown seaweed meals. Fishes fed with Diet A (control feed) were fed with conventional feed (Blue crown feed), while Diet B fishes were fed with 100% *Grasshopper meal*, Diet C fishes were fed with 50% fish feed, 50% Grasshopper and brown seaweed meal. While Diet D fishes were *fed with 100% fish meal*. From table 1, the fish fed with Diet A the bacteria isolate identify are *Streptococcus pyogenes, Enterobacter aerogene, Staphylococcus aureus, Shigella flexneri*, *Escherichia coli, Leuconostoc latis* and *Klebsella pneumonia*. The bacteria isolates found in the gastrointestinal tract of fish fed with diet B are *Bacillus subtilis, Lactococcus bulgaricus, Lysinibacillus macroides,* and *Lysinibacillus capsici, Stenotrophomonas pavanii* and *Lysinibacillus* sp. In the fish fed diet C, *Bacillus subtilis, Lactococcus bulgaricus, Lysinibacillus* sp., *Staphylococcus aureus, Lysinibacillus macroides, Stenotrophomonas pavanii* and *Enterobacter aerogene* where the bacteria isolates found in the GIT of of the fish. While the bacteria isolate in gastrointestinal tract of fish fed with diet D were *Bacillus cereus, Staphylococcus aureus, Pedicoccus acidilactis, Escherichia coli, Salmonella typhi, Leuconostoc latis* and *Shigella flexneri.* In term of bacteria diversity in the gastrointestinal tract,

fishes fed with Diet A, C and D recorded highest with seven (7) species, while fish fed with diet B recorded six (6) species of bacteria each in term of diversity. Summary of each bacterial isolate presence in the gastrointestinal tract of fish are shown in Table 3.

3.2 Genotypic identification

Genotypic identification: The Polymerase chain reaction (PCR) products obtained from the selected isolates following amplification and sequencing with the 16S rRNA were estimated at 1000bp in size (Plate 1). The percentage similarity of the nucleotide sequence of selected seven bacteria isolates are shown in Table 4.

The gene sequence of sample 1 strains was blast and compared with NCBI gene bank database and the homology shows 98.02% maximum identity with that of *Lysinbacillus capsici* strain with NCBI accession number NZ_UAQE01000001.1Sample 2 homology shows 99.47% maximum identity with that of *Stenotrophomonas pavanii* strain with NCBI accession number of NZ_APO24684.1.Sample 3 strains homology shows 88.91% maximum identity with that of *Alcaligenes faecalis* strain with NCBI accession number of NZ_CPO13119.1. Sample 4 homology shows 81.68% maximum identity with that of *Klebsiella pneumoniae* strain with NCBI accession number of NC_016845. Sample 5 gene sequence homology shows 90.37% maximum identity with that of *Bacillus cereus* strain with NCBI accession number of NZ_CP072774.1. The gene sequence of sample 6 shows homology of 98.02% maximum identity with that of *Lysinibacillus macroides* Strain with NCBI accession number of MN249381 and Sample 7 gene sequence homology shows 83.40% maximum identity with that of *Lysinibaccillus subtilis* strain with NCBI accession number of MT968435_1. The sequences were further aligned and phylogenetic trees for the isolates sequence strains were determined using the neighbor-joining method to know the genetic relatedness between all bacteria isolates strain encountered.

Discussion

The demand for fish and other aquaculture products is increasing globally and has resulted in advancement in term of fish feed and nutrition, which function as catalysts for the long-term growth of the rapidly increasing aquaculture sector (Wanka *et al*., 2018). Probiotic dietary

supplements have been shown to improve fish health and nutrition; however, these bacteria are mostly obtained from some terrestrial and warm-blooded hosts which limit their effectiveness in fish (Hortillosa *et al.,* 2022). Most probiotics bacteria found in the GIT system of the relevant fish species will colonize more quickly within the main host (Wanka *et al*., 2018). Bacteria are numerous in nature, which may contribute to their abundance in the habitat where fish dwell (Mohammed and Adamu, 2019), making it hard to avoid being part of their diet. Bacteria found their way into fish diet during preparation, consumption and may adapt in the gastro intestinal system and develop a symbiotic connection within the fish digestive tract (Jimoh *et al*., 2014). Bacteria presence in the GIT of fish have been reported in fish diet containing insect protein (Adamu *et al.,* 2022) and has been observed to be substantially greater than in surrounding water; which is an indication that the digestive tracts of fish provide suitable habitat for their growth (Sakata, 1990; Jimoh *et al.*, 2014). The presence of some facultative pathogen such as *Escherischia coli*, *Salmonella* sp., *Streptococcus* sp. and *S. aureus* in the control feed which under unfavorable condition could results to disease in fish (Adamu *et al*., 2021a) as they have been implicated to be fish borne and causes infectious diseases in fish (Amande and Nwaka, 2013). In aquaculture, *Pseudomonas* sp and *Enterobacter aerogenes* have been found to be an opportunistic pathogen (Amande and Nwaka, 2013). They have had been isolated in wounds, burns, eyes, ear infections and urinary infection in humans (Amande & Nwaka (2013). The presence of *Salmonella sp* is also an opportunistic pathogen in aquaculture, the species are also associated with food spoilage, *Salmonellosis* resulting in bactereamia and severe typhoid in human or salmonella fever (Egbere *et al.,* 2010). Similarly, *Escherischia coli* and *Shigella* have been reported to be causative agent of gastroenteric disease such as diarrhea, vomiting, fever, colitis heamolytic urinary syndrome with renal failure. *Streptococcus pyogenes* have also been reported to causes pancreatic disease and eye lesion in fish respectively (Whalen, 2007). *Klebsiella pneumoniae* also inhabits the gastrointestinal tract of fish and causes infection wound at adverse level (Tan *et al.,* 2014). Most of these opportunistic bacteria species have been reported to be natural microflora in GIT of fish and their presence in the experimental pond could be due to contamination through handling of the feed or water source which are related to fecal deposit (Rhu *et al*., 2010).

The prevalence of probiotic species in the GIT of fishes fed supplemented Diet B, C and D as compare to Diet A which is the conventional feed has shown that the supplementary diet which is of insect nature and fish meal contain probiotic which can improve the diet and fish growth.

The probiotic bacteria are identified as those isolates of bacteria from *Bacillus*, *Lactobacillus* and *Lactococcus* species. This is in conformity with the work of Agustina *et al.*, (2022) who reported that bacteria species from *Lactococcus*, *Enterococcus* and *Lactobacillus* groups exhibit some antibacterial activity in GIT tract of fishes in the strong range. Studies have shown that this groups of bacterial improves the quality of the water by influencing the microbial communities in fish intestinal tract and the fish culturing environment; as it is a good probiotic candidate for improving the aquaculture in terms of water quality and feed (Wang *et al*., 2020). Similarly, *Lysinibacillus* sp and *L. macrolides* reported in D4 and D1 are also probiotic bacteria (Adamu *et al.,* 2022). Rahman *et al*., (2014) have reported that this groups of bacterial shows potential for bioremediation in aquaculture.

The prevalence and abundance of probiotics in GIT of fish fed with Diet B and C as compare to their absence in the gastrointestinal tract of the fishes fed with Diet A is an indication that insect protein (especially grasshopper protein) is of good and quality feed and can be used to supplement fish meal in feed diet of fishes. The probiotics bacteria presence in the supplementary feed has the capacity to increase the health status as well as growth performance of the fish (Wanka *et al*., 2018; Yang *et al*., 2022; Hortillosa *et al*., 2022).

Conclusion

This study established the efficacy of hybrid catfish fed with supplemented grasshopper meal by isolating probiotic bacteria in gastro intestinal tract showing comparative advantages as the insect protein decreased in the diets. The presence of probiotics bacteria in GI tract of hybrid catfish fed Diet B, Diet C and Diet D could be associated with the diets and insect protein present in the feed.

Table 2: Biochemical characteristic of bacterial isolates from gastrointestinal tract of Hybrid catfish fed supplemented Grasshopper meal

Diet	Colony morphology	Media	Gram	Shapes	Indole	Catalase	Citrate	Urease	Coagulase	Oxidase	Glucose	Lactose	Sucrose	Suspected bacteria
A	Greyish black	NA	+	Cocci	-	+	+	-	+	-	A	A	A	*Streptococcus pyogenes*
	Pink colonies	MCK	-	Rod	-	+	+	-	+	-	AG	AG	AG	*Enterobacter aerogenes*
	Brown to black colonies	EMB	+	Cocci	-	+	+	+	+	-	A	A	A	*Staphylcoccus aureus*
	Transparent and colorless	EMB	-	Rod	+	+	-	-	-	-	AG	NA	NA	*Shigella flexneri*
	Blue-black, with metallic sheen	EMB	+	Rod	+	+	-	-	-	-	AG	AG	AG	*Escherichia coli*
	Slimy, smooth, round grayish	MRS	+	Cocci	-	-	-	+	+	-	A	AG	NA	*Leuconostoc latis*
	Rose pink	EMB	-	Rod	-	+	+	-	-	-	AG	AG	AG	*Klebsella pneumonia*
B	Grey white colonies	NA	+	Rod	-	+	+	-	-	+	AG	NA	A	*Bacillus subtillis*
	Creamy grey colonies	MRS	+	Cocci	-	-	-	-	-	-	A	A	NA	*Lactobacillus bulgaricus*

	Whitish grey	NA	+	Rod	-	-	-	+	-	+	AG	NA	A	*Lysinibacillus macrolides*
	Creamy whitish grey	NA	+	Rod	-	+	+	+	+	+	AG	NA	AG	*Lysinibacillus capsici*
	Yellowish smooth and circular	EMB	-	Rod	-	+	+	-	+	-	A	NA	A	*Stenotrophomonas pavanii*
	Whitish grey	NA	+	Rod	-	+	+	+	+	+	AG	NA	AG	*Lysinibacillus* sp
C	Grey white colonies	NA	-	Rod	-	+	+	+	-	+	AG	A	A	*Bacillus cereus*
	Brownish colonies	MRS	+	Cocci	-	-	-	-	-	-	A	A	NA	*Lactobacillus. bulgaricus*
	Whitish grey	NA	+	Rod	-	+	+	+	+	+	AG	NA	AG	*Lysinibacillus* sp
	Brown to black colonies	EMB	+	Cocci	-	+	+	+	+	-	A	A	A	*Staphylcoccus aureus*
	Whitish grey	NA	+	Rod	-	-	-	+	-	+	AG	NA	A	*Lysinibacillus macroides*
	Yellowish smooth and circular	EMB	-	Rod	-	+	+	-	+	-	A	NA	A	*Stenotrophomonas pavanii*
	Pink colonies	MCK	-	Rod	-	+	+	-	+	-	AG	AG	AG	*Enterobacter aerogenes*
D	Grey white colonies	NA	-	Rod	-	+	+	+	-	+	AG	A	A	*Bacillus cereus*
	Brown to black colonies	EMB	+	Cocci	+	+	-	-	-	-	AG	A	A	*Staphylococcus aureus.*
	Slimy, smooth, round grayish	MRS	-	Rod	-	-	-	-	+	-	AG	NA	AG	*Pedicoccus acidilactici*
	Bright pink to red colonies	MCK	+	Rod	+	+	-	+	-	-	AG	AG	AG	*Escherichia coli*
	Brown to blue black	EMB	-	Rod	-	+	-	-	-	-	AG	AG	NA	*Salmonella typhi*

Slimy, smooth, round grayish	MRS	+	Cocci	-	-	-	+	+	-	A	AG	NA	*Leuconostoc latis*
Transparent and colorless	EMB	-	Rod	+	+	-	-	-	-	AG	NA	NA	*Shigella flexneri*

KEY: + = Positive; - = Negative; AG= Acid and Gas production; A= Acid production; NA = No Acid and Gas production

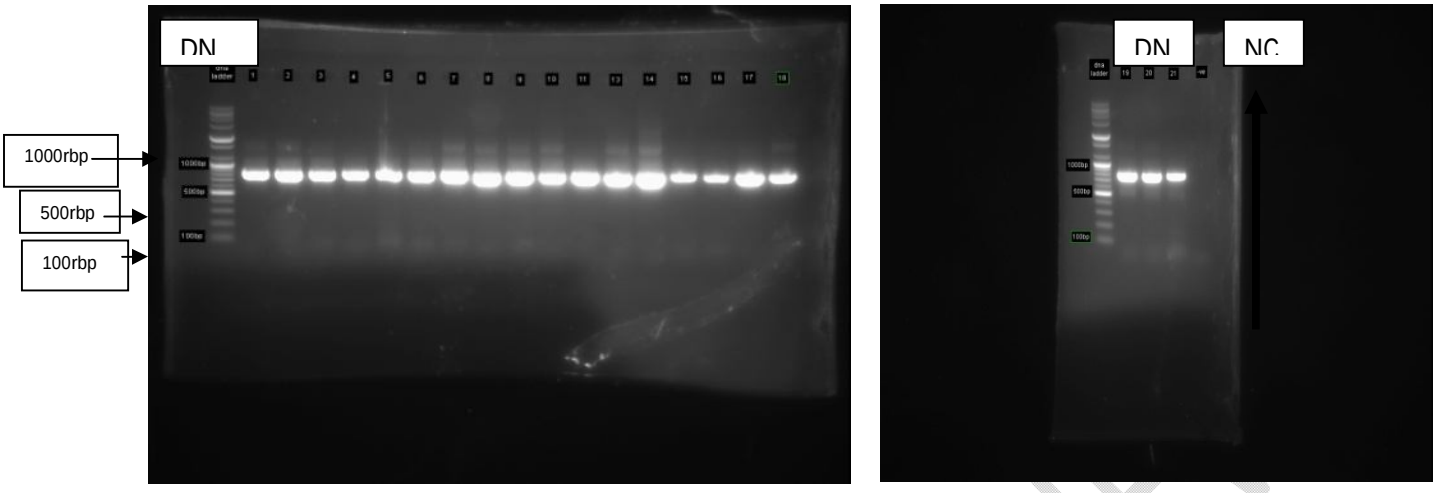
Table 3: Summary of the isolated bacteria species isolated from gastro-intestinal tract of fish fed grasshopper supplemented diet

		Diet			
S/NO	Species	Diet A	Diet B	Diet C	Diet D
1	*Streptococcus pyogenes*	+	-	-	-
2	*Staphylococcus aureus*	+	-	+	+
3	*Escherichia coli,*	+	-	-	+
4	*Leuconostoc latis*	+	-	-	+
5	*Bacillus subtilis*	-	+	-	-
6	*Lactobacillus bulgaricus*	-	+	+	-
7	*Lysinibacillus capsica*	-	+	-	-
8	*Lysinibacillus macrolides*	-	+	+	-
9	*Lysinibacillus* sp	-	+	+	-
10	*Enterobacter aerogene*	+	-	+	-
11	*Shigella flexneri*	+	-	-	+
12	*Bacillus cereus*	-	-	+	+
13	*Klebsella pneumonia*	+	-	-	-
14	*Stenotrophomonas pavanii*	-	+	+	-
15	*Pedicoccus acidilactis*	-	-	-	+
16	*Salmonella typhi.*	-	-	-	+

Note: += present, -= absent.

Table 4. Similarity percentage of bacteria isolates.

Sample	Similarity (%)	Nearest relatives	Accession number of nearest relatives in NCBI database
1	98.02	*Lysinibacillus capsici*	NZ_UAQE01000001.1
2	99.47	*Stenotrophomonas pavanii*	NZ_APO24684.1
3	88.91	*Alcaligenes faecalis*	NZ_CPO13119.1
4	81.68	*Klebsiella pneumonia*	NC_016845
5	90.37%	*Bacillus cereus*	NZ_CP072774.1
6	98.02	*Lysinibacillus macroides*	MN249381
7	98.02	*Lysinibaccillus subtilis*	MT968435_1



Key: DN=DNA ladder, NC= Negative control, 1-21 products of bacteria isolates

Figure 1: Binding profile of bacteria isolates

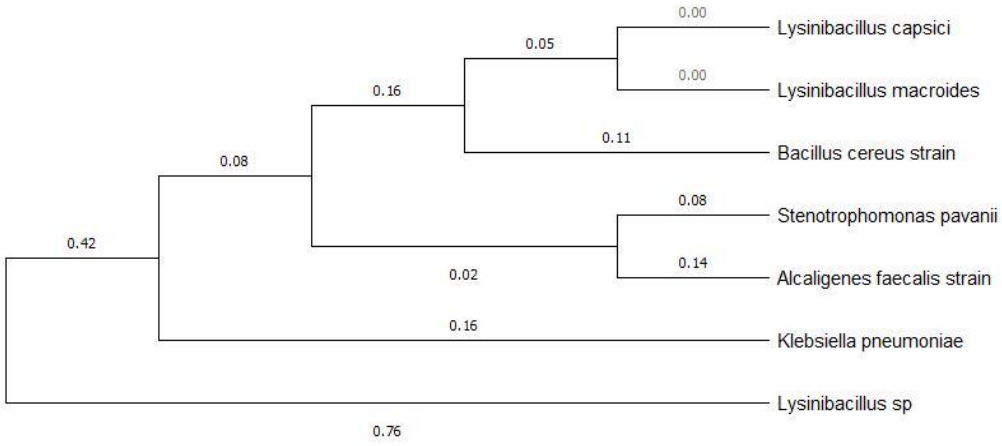


Figure 2. Phylogenetic tree of some bacteria isolates

Data availability statement

The data that support the findings of this study are available on request from the corresponding author.

Ethical approval statement.

This project was submitted and approved by research ethic committee of Ibrahim Badamasi Babangida University, Lapai Niger state with reference number IBBUL/01/2019

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