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Research Article

Pathogenicity Assay of Probiotic-potential Bacteria (*Bacillus* Species) on Live Catfish (*Clarias anguillaris*) Juveniles

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Abstract

Pathogenicity test is one key criterion used in selecting probiotics for use in food producing animals. This experiment was aimed to ascertain the safety of 10 selected probiotic-potential *Bacillus* species (Bsp). Three hundred and sixty *Clarias anguillaris* juveniles were obtained from homestead fish ponds within Makurdi metropolis. The fingerlings were distributed in 10 experimental groups: Bsp1, Bsp2, Bsp3, Bsp4, Bsp5, Bsp6, Bsp7, Bsp8, Bsp9 and Bsp10 and 2 control groups viz: positive control (PC) and negative control (NC). Each group was assigned 10 fingerlings in replicate. The PC group received 0.2 x 10^8 $CFUml^{-1}$ of pathogenic bacteria *Vibrio alginolyticus*, the NC received 0.2 mls of PBS and test groups received 0.2 x 10^8 $CFUml^{-1}$ *Bacillus* strains. The groups were observed for 20 days for morbidity and/or mortality from respective test groups. Survival rate of 60% (PC), 70% (Bsp8), 80% (Bsp6), 90% (Bsp2) whereas 100% were recorded for the rest of the groups. The weight gain of the PC group was significantly lower ($P \leq 0.05$) than all groups except for Bsp6. Also, Bsp7, recorded highest weight gain (20.82 ± 8.2 g) whereas Bsp1, Bsp2, Bsp4, Bsp5, Bsp8, Bsp9 and Bsp10 were significantly higher compared to both PC and NC. All physico-chemical parameters were within the reference interval (RI) for catfish. The 100% survival from Bsp1, Bsp3, Bsp4, Bsp5, Bsp7, Bsp9, and Bsp10 compared to PC were signs that these *Bacillus* strains were not pathogenic to the fish used, whereas Bsp2, Bsp6 and Bsp8 were mildly pathogenic to the experimental fish, though environmental factors could be incriminated. The high weight gain by Bsp7 (20.82 ± 8.30), Bsp1 (17.86 ± 4.24), Bsp2 (14.48 ± 1.65), Bsp4 and Bsp10 respectively (13.94 ± 4.80 and 13.36 ± 4.36) showcased the growth stimulation potentials in these isolates. The present study, showed that survival, growth performance, and regulation of physico-chemical parameters were significantly ($P \leq 0.05$) high with Bsp7, Bsp1, and Bsp10, so can be regarded as safe and can improve growth performance in fish production. These 3 *Bacillus* strains were identified as *B. subtilis* (MN099359.1), *B. subtilis* MK085082.1 and *B. velezensis* (CP041145.1).

Keywords

Probiotics, *Bacillus* strains, Pathogenicity, Assay, Catfish, Juveniles

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1. Introduction

Fish is the major component of aquaculture and the fastest growing food-protein producing sector in the world [14, 16, 34], but diseases have been reported as a major constraint [33] and greatest threat to aquaculture farms [34]. The effects of diseases are many, which include morbidity, mortality, reduced growth rate, and increased cost of production which pose serious setbacks for the continued growth of fishery industry [26]. Bacterial diseases, especially those caused by Gram-negative organisms, are responsible for mass mortality in both wild and cultured aquatic organisms [1].

Maintenance of good health is critical to a profitable fish production and the best way to manage the health is through disease prevention. The indiscriminate use of antibiotics as a way of corrective measures hardly yield success [25] and has attracted a global attention due to development of antibiotic resistance and drug residues in the meat. In many countries of the world this has led to restrictions on the use of some antimicrobial agents such as tetracycline [9] in food producing animals in view of the public health implications [11, 21, 32].

In Nigeria, despite the fact that aquaculture industry is expanding as well as regular use of antibiotics, the level of restriction on antibiotic use generally appears to be insignificant; while the problems associated with indiscriminate use of drugs have been reported in terms of antibiotic resistance [25, 26]. In view of this problem, there is a serious need for both pro-active and reactive approaches to control these problems through the development of biological agents (probiotics) in aquaculture that will effectively reduce or replace the use of antimicrobial agents.

The advocacy of choice for fish disease control has been probiotics due to its safety in terms of development of resistance and drug residue in food animals [31]. The future of probiotics and their importance in achieving and maintaining good health holds generally substantial promises. Probiotics have several advantages over the conventional antibiotics which include: improvement of nutrition by detoxification of potentially harmful component in feed, denaturing of potentially indigestible component in the diet by digestive enzymes (amylases and proteases), production of vitamins such as biotin and vitamin B_{12}, production of inhibitory compounds and stimulation of host immunity [19].

The dominant groups of probiotics that are used in fish culture belong to Gram positive bacteria, especially lactic acid bacteria, *Bacillus*, *Streptobacillus*, and *Bifidobacteria* groups [7]. On the other hand, some Gram-negative bacteria such as *Aeromonas hydrophila, A. sobria, Pseudomonas species, Vibrio sp* and *Enterobacteria* have probiotic potential [2], and so also fungi such as *Saccharomyces cerevisiae* [8]. All these bacteria differ greatly in their mode of action including the ability to modulate immune systems. Therefore, every probiotic differs from each other by their functional role. It is recognized that each strain has unique properties and the probiotic effects of a specific strain must not be extrapolated to other strains [3]. Of all bacterial genera used as probiotics in both terrestrial and aquatic environment, *Bacillus* species have been reported to have a wider range of action in human, animal and aquatic environment [24, 28]. The Candidacy of a probiotic depends on the ability of the bacterial cells or their spores to survive and grow at the high acidic environment of the stomach and the detergent-like activity of intestinal bile salts. *Bacillus* species offers higher acid tolerance and better stability during heat processing and low temperature storage. Several *Bacillus* strains have been screened for their potential probiotic functionality in several *In-vitro* and *In-vivo* models and most of the strains do carry probiotic attributes [15]. The objective of this was to ascertain the safety of the *Bacillus* species isolated and identified to the Catfish (*Clarias anguillaris*).

2. Materials and Methods

2.1. Experimental Fish and *Bacillus* Strains

A complete randomized experimental design was employed in this study. Three hundred and sixty *Clarias anguillaris* juveniles obtained from a homestead fish farmer in Makurdi metropolitan and transported to the experimental station. After acclimatizing the fish for 2 weeks, they were distributed into 12 groups viz: Bsp1, Bsp2, Bsp3, Bsp4, Bsp5, Bsp6, Bsp7, Bsp8, Bsp9 Bsp10, positive control (PC) and negative control (NC) each comprising 10 fish in triplicate. The pathogenicity test was carried out according to the modified protocol of Edward *et al.* [13, 30]. All the fish in PC group were injected intramuscularly with 0.2 mL of potential fish pathogen *Vibrio alginolytius* at the concentration of 1 x 10^8 CFU/mL that was estimated with 0.5 McFarland standards. All the fish in NC group were injected intramuscularly with 0.2 mL PBS whereas all the fish in test groups (Treatments 1-10) were injected intramuscularly with 0.2 mL of different test *Bacillus* strains respectively at the concentration of 1 x10^8 CFU/mL^{-1}. The test groups were according to the number of successful *Bacillus* strains selected through the screenings for probiotic properties using standard procedures.

After injection, all the groups of fish (10 fish per replicate) were kept in a round bottomed plastic basin of 50-liter capacity and were observed for any morbidity or mortality for 20 days. During this period, the fish were given commercial pelleted fish feed (Copens®, Thailand) and were fed twice daily. The culture water was changed 100% every 5 days, since there was no provision of artificial aerators.

2.2. Physico-chemical Parameters of Rearing Water

Five physio-chemical parameters were measured during this study, which includes; temperature (T^o), pH, dissolved oxygen (DO), total dissolved solids (TDS), and electrical

conductivity (EC). The T^{o} and DO concentration were measured using the Traceable Dissolved oxygen meter. This was done by lowering the meter in to the water and allowed for 3 minutes to stabilize before readings were done. The pH, TDS, EC were measured using the HANNA GROCHEK meter. These were achieved by lowering the meter into the water and wait for 3 minutes when there was stability, the reading was taken. These Physico-chemical parameters of the rearing water were measured on day 0, 4, 8, 12, 16, and 20. Data generated were subjected to One Way Analysis of Variance (ANOVA) using the Statistical Package of Social Sciences (SPSS) version 21. Significance was accepted at the probability level of 95% ($P \leq 0.05$). The variant means were separated by Duncan's Multiple Range Test.

2.3. Confirmatory Identification of *Bacillus* Species

Following successful screening and pathogenicity trials, the isolates were subjected to molecular identification. After the extraction of the genomic DNA, amplification of the 16 S rDNA gene was carried out on four successful *Bacillus* species by PCR using universal primers 27 F and 1492R targeting the V1 to V9 variable regions followed by sequencing (sense and anti-sense) of the 1465 bp amplified products using primers 27 F, 1492 R, 518 F and 800 R as previously described by Rahman *et al.* [27]. A consensus sequence covering the entire amplified region was then assembled using the Bio-Edit Software (STABvida, Portigal). Identification of each isolate was carried out by querying each consensus sequence to sequences in the GenBank using the basic local alignment search tool (BLAST). The most similar bacterial species was found in the GenBank by using BLAST search.

3. Results

The results of pathogenicity assay of the potential probiotic *Bacillus* strains and weight gain are presented in the Table 1 below:

Table 1. *Survival rate and weight gain of Clarias anguillaris juveniles treated with probiotic potential Bacillus strains.*

Treatment	Survivability (%)	Initial weight (g)	Final weight (g)	Weight gain (g)
PC	60	10.40 ±2.88^{ab}	17.68 ±8.75^{b}	07.28 ±5.87
NC	100	11.44 ±2.92^{ab}	22.14 ±9.09^{ab}	10.70 ±6.17
Bsp1	100	7.9 ±4.07^{bc}	25.76 ±8.28^{ab}	17.86 ±4.24
Bsp2	90	3.9 ±2.2^{d}	18.38 ±3.85^{b}	14.48 ±1.65
Bsp3	100	13.82 ±3.28^{a}	23.02 ±4.08^{ab}	09.20 ±0.80
Bsp4	100	9.32 ±2.8a^{bc}	23.26 ±7.60^{ab}	13.94 ±4.80
Bsp5	100	12.8 ±3.0^{a}	21.02 ±9.54^{ab}	08.22 ±6.56
Bsp6	80	9.3 ±4.6^{abc}	15.18 ±4.97^{b}	05.88 ±0.37
Bsp7	100	10.46 ±4.2^{ab}	31.28 ±12.50^{a}	20.82 ±8.30
Bsp8	70	4.9 ±2.8^{cd}	15.52 ±3.29^{b}	10.62 ±0.49
Bsp9	100	4.32 ±1.75^{cd}	15.18 ±4.97^{b}	10.86 ±3.22
Bsp10	100	6.76 ±4.28^{bc}	20.12 ±8.64^{ab}	13.36 ±4.36

Values are mean ± SD, n = 30, values with different alphabet superscript are significant at $P \leq 0.05$, PC = positive control, NC = negative control and Bsp = *Bacillus* strains

The result showed that PC recorded the least survival rate (60%), followed by Bsp8 (70%) and Bsp9 (80%). Bsp2 had 90% survival rate and all the rest recorded 100% survival. There was significant difference ($P \leq 0.05$) in the growth rate of Bsp7 when compared with other groups. The body weight of the treated fish groups Bsp6, Bsp7 and Bsp8 were lower, but not significantly different ($P \geq 0.05$) from the PC. However, Bsp1, Bsp3, Bsp4, Bsp5, Bsp10 and NC were significantly ($P \leq 0.05$) different from the PC. These significant differences were evident with high weight gain of these fish groups compared to the PC. Bsp7 recorded the highest weight gain in 20 days followed by Bsp1, Bsp2 and Bsp4. Bsp6 recorded the least weight gain of 5.88 ± 0.37, even lower than the PC, although there 20% mortality. The growth performance in Bsp1, Bsp2, Bsp4, Bsp7 and Bsp10 were higher compared to the NC.

Table 2, presents the result of temperature and pH of the culture water. The temperature ranged between 24.47 to

33.07 ℃ throughout the experimental period. The acceptable range of temperature for catfish has been 25 to 32 ℃. The temperature was found within the reference interval (RI) for catfish production except in PC at day 16 which was significantly higher than NC. At day 20, Bsp1 and the PC significantly ($P \leq 0.05$) recorded higher values compared to other treatments.

Similarly, the pH levels were found to fall within the reference interval of 6.5 to 8.5 (Table 2). The pH level in this study ranges from 7.13 to 9.47. At day 20, Bsp7, 8, and 9 recorded significantly ($P \leq 0.05$) lower pH than both PC and NC. It was observed that PC, recorded ($P \leq 0.05$) high values throughout the experiment.

Table 2. *The mean temperature and pH values of the rearing water in 20 days experiment.*

Treatment	Average Temperature (RI: 25-32 ℃) Mean ± SD	pH level (RI: 6.5-8.5) Mean ± SD
PC	29.00 ± 1.47	8.78 ± 0.40
NC	28.74 ± 0.69	8.30 ± 0.31
Bsp2	28.54 ± 1.22	8.05 ± 0.15
Bsp3	28.22 ± 0.20	7.84 ± 0.14
Bsp4	28.22 ± 0.33	7.81 ± 0.16
Bsp5	28.06 ± 0.41	7.83 ± 0.14
Bsp6	27.77 ± 0.48	7.72 ± 0.25
Bsp7	27.72 ± 0.33	7.75 ± 0.15
Bsp8	27.83 ± 0.75	7.68 ± 0.19
Bsp9	27.89 ± 0.56	7.61 ± 0.39
Bsp10	27.79 ± 0.51	7.72 ± 0.22

Values are mean ± SD, n = 30, PC = positive control, NC = negative control and Bsp = *Bacillus* strains

The result of DO presented in Table 3 were found to be between the RI of 5 to 10 mg/L. There was steady decrease in values from day 0 of the test to day 20. At day 0 the value ranged 2.53 to 3.37, while at day 20 the range fall within 1.40 to 1.77 mg/L. The values of DO decreases as the days of experiment increases.

Table 3. *Dissolved oxygen (DO) of rearing water of C. anguillaris during pathogenicity assay.*

Treatment	Days of experiment (RI: 5 – 10 mg/L)					
	0	4	8	12	16	20
PC	3.73 ± 0.15^{a}	3.30 ± 0.10^{b}	3.77 ± 0.06^{a}	2.13 ± 0.15^{ab}	1.70 ± 0.10	1.77 ± 0.15
NC	3.00 ± 0.26^{bc}	3.40 ± 0.10^{b}	3.03 ± 0.06^{b}	2.47 ± 0.06^{ab}	1.50 ± 0.10	1.60 ± 0.10
Bsp1	3.20 ± 0.10^{b}	3.27 ± 0.15^{b}	3.03 ± 0.06^{b}	2.17 ± 0.64^{ab}	1.23 ± 0.23	1.90 ± 0.10
Bsp2	3.10 ± 0.10^{bc}	3.80 ± 0.10^{a}	3.03 ± 0.06^{b}	2.80 ± 0.10^{a}	1.23 ± 0.25	1.63 ± 0.06
Bsp3	2.53 ± 0.40^{a}	3.17 ± 0.06^{b}	2.33 ± 0.06^{c}	1.93 ± 0.35^{ab}	1.77 ± 0.15	1.53 ± 0.30
Bsp4	2.63 ± 0.21^{a}	3.00 ± 0.06^{c}	3.10 ± 0.00^{b}	2.33 ± 0.21^{ab}	1.70 ± 0.10	1.60 ± 0.30
Bsp5	3.00 ± 0.10^{bc}	2.70 ± 0.10^{c}	2.10 ± 0.34^{d}	1.90 ± 0.90^{b}	1.47 ± 0.49	1.63 ± 0.21
Bsp6	3.10 ± 0.10^{bc}	2.00 ± 0.16^{d}	1.73 ± 0.06^{d}	2.07 ± 0.47^{ab}	1.03 ± 0.15	1.50 ± 0.10

Treatment	Days of experiment (RI: 5 – 10 mg/L)					
	0	4	8	12	16	20
Bsp7	3.33 ± 0.21^{b}	2.00 ± 0.10^{d}	1.93 ± 0.06^{d}	1.97 ± 0.74^{ab}	1.43 ± 0.11	1.47 ± 0.06
Bsp8	3.00 ± 0.10^{bc}	1.73 ± 0.67^{c}	1.80 ± 0.10^{d}	1.97 ± 0.50^{ab}	1.30 ± 0.62	1.40 ± 0.10
Bsp9	2.80 ± 0.10^{cd}	1.90 ± 0.10^{d}	1.40 ± 0.26^{d}	1.80 ± 0.10^{b}	1.60 ± 0.60	1.50 ± 0.61
Bsp10	3.37 ±0.21^{b}	2.00 ±0.11^{d}	1.43 ±0.06^{d}	1.73 ±0.15^{b}	1.67 ±0.68	1.50 ±0.43

Values are mean ± SD, n = 30, values with different alphabet superscript are significant at P ≤ 0.05, PC = positive control, NC = negative control and Bsp = *Bacillus* strains

The result of TDS presented in Table 4 showed a steady increase in values from day 0 to day 20, and all the values were found within acceptable range for catfish of 50 to 5000 ppm. The range recorded in this study was between 50.33 ± 8.08 to 381.00 ±26.85 ppm. Bsp10 recorded significantly (P ≤ 0.05) high values at day 12, 16 and 20 compared to both PC and NC.

Table 4. *The TDS of rearing water of C. anguillaris during pathogenicity assay.*

Treatment	Days of Experiment (RI: 50-5000 ppm)					
	0	4	8	12	16	20
PC	50.33 ±8.08^{d}	147.33 ±0.58^{e}	191.67 ±1.33^{de}	252.67 ±3.21^{b}	294.33 ±4.04^{de}	319.67 ±17.04^{ab}
NC	56.00 ±6.08^{cd}	138.67 ±0.58^{e}	189.00 ±1.00^{de}	243.00 ±6.08^{bcd}	326.67 ±23.09^{bc}	312.33 ±10.78^{cd}
Bsp1	65.00 ±2.00^{cd}	137.00 ±1.00^{e}	190.33 ±1.53^{de}	255.00 ±4.36^{b}	305.33 ±4.51^{cd}	337.67 ±6.30^{b}
Bsp2	71.00 ±1.00b^{c}	145.00 ±1.00^{d}	191.33 ±0.58^{de}	235.00 ±1.09^{cd}	280.00 ±17.32^{de}	287.00 ±11.26^{e}
Bsp3	97.00 ±25.12^{a}	131.67 ±1.00^{e}	167.33 ±0.58^{f}	211.00 ±7.94^{e}	234.67 ±1.53^{f}	247.33 ±2.08^{f}
Bsp4	103.67 ±19.50^{a}	123.67 ±1.53^{e}	160.33 ±0.58^{de}	198.00 ±1.00^{e}	233.67 ±6.08^{f}	256.67 ±5.77^{f}
Bsp5	70.67 ±1.15^{bc}	158.00 ±1.00^{e}	201.33 ±2.08^{b}	236.33 ±31.46^{cd}	270.00 ±1.00^{e}	314.67 ±12.86^{cd}
Bsp6	87.33 ±2.08^{a}	143.00 ±1.00^{d}	192.33 ±6.65^{de}	233.33 ±0.58^{c}	274.33 ±3.78^{e}	296.00 ±5.29^{de}
Bsp7	87.33 ±1.53^{ab}	141.67 ±2.52^{d}	195.33 ±2.08^{cd}	238.33 ±1.53^{bcd}	287.67 ±6.80^{de}	300.00 ±2.08^{cde}
Bsp8	69.00 ±1.00^{bc}	221.00 ±2.00^{a}	266.33 ±0.51^{a}	241.33 ±3.21^{bcd}	338.33 ±37.33^{ab}	315.00 ±5.00^{cd}
Bsp9	71.33 ±1.32^{bc}	155.00 ±2.64^{c}	200.00 ±1.00^{ab}	239.00 ±1.00^{bcd}	270.00 ±1.00^{e}	293.67 ±3.21^{de}
Bsp10	69.00 ±1.00^{bc}	166.33 ±5.50^{b}	203.67 ±2.31^{b}	328.67 ±0.56^{a}	355.00 ±18.03^{a}	381.00 ±26.85^{a}

Values are mean ± SD, n = 30, values with different alphabet superscript are significant at P ≤ 0.05, PC = positive control, NC = negative control and Bsp = *Bacillus* strains

Similarly, there was a steady increase in the EC from day 0 to day 20 (Table 5). From day 12, the values recorded were beyond the acceptable range of 30 to 500 µScm^{-1}. Bsp10, had significantly (P ≤ 0.05) high values on day 12, while Bsp8, 9 and 10 had high values of EC respectively on day 16 and Bsp1 recorded highest values of EC on day 20. All these values were significantly (P ≤ 0.05) higher compared to both the values of PC and NC.

Table 5. *Electrical conductivity of rearing water of C. anguillaris during pathogenicity assay.*

Treatment	Days of experiment RI: (30-500 µS/cm)					
	0	**4**	**8**	**12**	**16**	**20**
PC	103.00 ±18.68^{d}	300.00 ±1.00^{d}	386.67 ±1.53^{bcd}	504.33 ±3.78^{b}	574.33 ±22.05^{bc}	651.33 ±7.09^{bc}
NC	113.33 ±12.42^{cd}	279.00 ±1.00^{d}	350.00 ±5.00^{ef}	481.00 ±4.35^{cde}	603.33 ±18.92^{b}	621.33 ±18.58^{cde}
Bsp1	125.00 ±2.00^{cd}	276.00 ±1.00^{d}	376.00 ±5.29^{ef}	505.00 ±4.35^{b}	603.33 ±10.11^{b}	679.33 ±9.02^{b}
Bsp2	141.00 ±1.00^{bc}	281.00 ±1.00^{d}	381.00 ±1.00^{def}	465.00 ±3.67^{e}	525.67 ±22.27^{c}	548.00 ±17.09^{f}
Bsp3	194.00 ±49.38^{a}	262.67 ±6.42^{d}	334.33 ±3.78^{ef}	414.33 ±2.08^{e}	456.67 ±23.09^{d}	492.33 ±10.79^{f}
Bsp4	220.00 ±10.00^{a}	245.67 ±2.08^{d}	319.00 ±1.00^{f}	395.00 ±4.35^{f}	455.00 ±5.00^{d}	508.33 f ±11.37^{f}
Bsp5	141.00 ±1.00^{bc}	317.67 ±1.53^{c}	413.67 ±22.81^{b}	499.67 ±9.50^{bc}	510.67 ±22.94^{cd}	632.67 ±28.31^{cd}
Bsp6	166.67 ±1.53^{b}	280.33 ±1.53^{d}	364.33 ±21.07^{ef}	463.00 ±2.65^{e}	532.33 ±28.22^{c}	590.67 ±9.45^{e}
Bsp7	122.33 ±2.08^{cd}	285.67 ±3.21^{d}	389.67 ±1.53^{bcd}	472.67 ±2.51^{de}	565.67 ±19.14^{bc}	605.00 ±30.51^{de}
Bsp8	109.00 ±3.01^{d}	429.33 ±25.89^{a}	505.67 ±32.35^{a}	491.33 ±0.58^{bcd}	696.33 ±49.80^{a}	606.33 ±21.22^{de}
Bsp9	120.33 ±1.53^{cd}	316.00 ±1.00^{c}	401.67 ±0.58^{bcd}	479.33 ±0.58d^{e}	689.00 ±59.10^{a}	556.67 ±5.77^{f}
Bsp10	111.00 ±3.61^{cd}	339.67 ±0.58^{b}	410.33 ±0.58^{bc}	615.33 ±35.57^{a}	676.33 ±86.95^{a}	778.33 ±17.56^{a}

Values are mean ± SD, n = 30, values with different alphabet superscript are significant at P ≤ 0.05, PC = positive control, NC = negative control and Bsp = *Bacillus* strains

Result of Confirmatory Identification of Bacillus strains.

The result of the confirmatory identification is presented in Table 6 below. Five strains of *B. subtilis*, two of *B. cereus*, one of *B. amyloliquifaciens* and two *B. velezensis* all corresponded to 100% in the GenBank according to the BLAST search.

Table 6. *Bacillus species distribution and Percentage similarity of identified strains against reference strains in the GenBank.*

Sample ID	Suggested spp	Accession number	Identity percentage (%)
Bsp1	*Bacillus subtilis*	MK085082.1	100
Bsp2	*Bacillus subtilis*	CP026608.1	100
Bsp3	*Bacillus cereus*	MN122695.1	100
Bsp4	*Bacillus subtilis*	MN099359.1	100
Bsp5	*Bacillus subtilis*	MK085082.1	100
Bsp6	*Bacillus cereus*	MN122695.1	100
Bsp7	*Bacillus subtilis*	MN099359.1	100
Bsp8	*Bacillus velezensis*	CP041145.1	100
Bsp9	*Bacillus amyloliquefaciens*	MN099360.1	100
Bsp10	*Bacillus velezensis*	CP041145.1	100

4. Discussion

The 100% survival rates were recorded with 7 strains of the *Bacillus* isolates in this study indicated that these *Bacillus* strains were not pathogenic to the *C. anguillaris* juveniles used and so are considered safe for use in catfish production. The high weight gain recorded within the 20 days in Bsp7, Bsp1, Bsp2, Bsp4, Bsp10 and Bsp9 signified that these *Bacillus* strains are potential growth promoters [17-18].

The low mortality recorded in Bsp2, Bsp6 and Bsp8 in this study corroborated with the result obtained by Anyanwu *et al*. [5], who reported that the probiotic-potential bacteria from catfish (*Clarias* species) gut demonstrated low virulence in catfish juveniles with a survival rate of 85-90%, although, the survival rate recorded in this study was 70 - 100%. Environmental factor in this study could have contributed to the few mortalities in the groups since the values of physico-chemical parameters fell within the acceptable levels [4, 6, 12, 24]. Some factors that play a role in bacterial pathogenicity were the propagation speed of pathogen and host defense against mechanism pathogen. Some bacterial extracellular products such as leucosidine and haemolysin were able to induce lysis of the blood cells [29], and then the bacteria spread throughout the host body to several target organs. Bacteria also have several types of enzymes in their extracellular products such as casein, gelatinase, chitinase, collagenase, elastase, hyaluronidase and proteinase [10, 18] that are able to break down complex compounds into simpler forms so that the bacteria can easily enter and damage the host cells. These *Bacillus* strains as potential probiotic bacteria, may have positive contributory factors to the host recorded in this study. These bacteria have inhibited pathogenic bacteria during the vitro test in the previous screening tests and reported elsewhere [22-23]. From this study therefore, Bsp7, Bsp1, and Bsp10 were the three *Bacillus* strains that produced no mortality with high weight gain were considered as potential probiotic bacteria. The performances might be due to their probiotic potential resulting to good growth and high survivability.

Dissolved oxygen is one of the most important parameters when assessing water quality in aquatic systems because of the influence oxygen has on water. The amount of dissolved oxygen in water is limited by physical conditions like temperature and atmospheric pressure. Low DO levels are accountable for more fish kills in the aquaculture industry than other factors such as temperature, alkalinity, and salinity. The amount of oxygen that a fish consumes depends on its size, activity level (feeding and reproduction), type of fish, and the temperature of the water.

Water temperature has a tremendous impact on water density as it affects the growth of organisms. The mean value of temperature reading was constant throughout the period of the study 27 ℃ for both structured and unstructured water. The water temperature reading of this study were in line with WHO [35] which recommended 25 ℃ - 31 ℃ as temperature range for optimum growth and survival of catfish.

Optimal pH range of aquatic life is 6.5 – 8.5 and has been noted to be productive levels and recommended for fish culture. Chronic pH levels below 6.5 and above 8.5 may reduce fish productivity and can results to fish mortality. A pH reading below 4.5 indicates that there is strong mineral acidity, which is harmful to fish and difficult to neutralize. Electrical conductivity (EC) is a measure of how well a solution conducts electricity and is correlated with salt content. The higher the concentration of ions present, the higher the conductivity of water [20]. These *Bacillus* strains might play great part in regulating all physicochemical parameters of the water to remain within the optimal range for catfish production.

5. Conclusion

The present experiment shows that survival, growth performance and regulation of Physico-chemical parameters of water were significantly higher in Bsp7, Bsp1, Bsp4 and Bsp10 in that order which were identified as *B. subtilis* (MN099359.1), *B. subtilis* MK085082.1 and *B. velezensis* (CP041145.1) respectively. The pathogenicity profile reveals that all these *Bacillus* strains are potential probiotics and safe for catfish. The few mortalities recorded could be associated with environment and handling stress during samplings. The survival rate, growth performance and maintenance of water parameters were best with Bsp7, Bsp1, Bsp4 and Bsp10 in that order. Therefore, the addition of these strains to improve growth performance with high survival rate is recommended to increase fish production.

Abbreviations

ANOVA	Analysis of Variance
BLAST	Basic local Alignment Search Tool
Bsp	Bacillus Species
CFU	Colony Forming Unit
CP, MK, MN	Code for Accession Numbers, Portugal
DNA	Deoxyribonucleic Acid
DO	Dissolved Oxygen
EC	Electrical Conductivity
FAO	Food and Agricultural Organization
JOSTUM	Joseph Sarwuan Tarka University
NC	Negative Control
PC	Positive
PCR	Polymerase Chain Reaction
pH	Acidity or Alkalinity of Susbstance
rDNA	Recombinant DNA
RI	Reference Interval
SD	Standard Deviation
SPSS	Statistical Package of Social Sciences
TDS	Total Dissolved Oxygen
TETFUND	Tertiary Education Trust Fund
WHO	World Health Organization

Author Contributions

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Conflicts of Interest

The authors declare no conflicts of interest.

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