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Evaluation Of Probiotic Potential Of Bacillus Subtilis FFOS For Application In Relevant Industry

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Abstract – Probiotics are known to improve the health of the host. Several microorganisms including Bacillus *species* have been reported to have probiotic potentials. Therefore the paper aimed to assess *Bacillus subtilis* FFOS for probiotic potential for its application in relevant industry. To determine probiotic potentials, several test including antagonistic activity, bile resistance, hemolytic activity and acid tolerance assays were carried out. The organism, *Bacillus subtilis* FFOS was applied in agriculture (poultry and farming). In poultry, nutrient broth (3%) containing the test organism was supplemented in the chicken feed for 12weeks and the feed without supplement served as control. The growth of each chicken was determined every week using a weighing balance. In farming, 2-bean seeds were planted into 3 bottles containing loamy soil respectively. Plant in bottle 1 (Sample A) was grown with nutrient broth containing *Bacillus subtilis* FFOS; plant in bottle 2 (Sample B) was grown with nutrient broth alone and bottle 3(Sample C) grown with water alone and their growth rates were monitored daily until 12days. The obtained result revealed antimicrobial potential of *Bacillus subtilis* FFOS against all the test pathogens (*Escherichia coli, Salmonella* sp. and *Staphylococcus aureus*) but more with Escherichia coli (8-10mm). *Bacillus subtilis* FFOS tolerate bile up to 7% concentration (4.0x10^4 CFU/mL), showed no hemolysis and was able to withstand acidic conditions (pH 3) up to 97.5 %. The assayed organism significantly enhanced the chicken growth (4.01kg/12wks)($p<0.05$). The broth supplemented with *Bacillus subtilis* FFOS enhanced the plant growth more than that without supplement and the one grown with water alone. The plant grown with water alone have higher growth than that grown with broth alone. The obtained result revealed *Bacillus subtilis* FFOS; a probiotics, good chicken growth enhancer and plant growth promoter. Hence it can be used as probiotics and supplement in chicken feed and plant growth.

Keywords – Evaluation, *Bacillus subtilis* FFOS, Probiotics, Application, Supplement.

I. INTRODUCTION

Probiotics are live microorganisms which when administered to hosts in adequate amounts helps in improving the health of the host. These microorganisms have been developed for both human and animal consumption. They are used to fortify animal feed especially in the poultry, ruminant, pig and aquaculture [1]. They have shown their ability to promote plant growth and to suppress plant pathogens [2]. They are also used as biofertilizers, plant strengtheners, phytostimulators, and biopesticides [3].

Many microorganisms including *Lactobacillus*, *Bifidobacterium*, *Saccharomyces cerevisiae*, *Aspergillus niger*, and *Bacillus* sp. have been reported for their probiotics potential.

Bacillus spp. are used as probiotics due to their ability to form resistant spores under environmental stress [4]. They resist physical and chemical conditions such as high temperature or pressure, air-drying activity, and UV light [5]. In addition to spore formation, their growth rate rates is fast, can grow in anaerobic and aerobic conditions, and contain high level of enzyme production which is one of its unique characteristics [6], [7]. *Bacillus* spp. including *B. subtilis*, *B. polyfermenticus*, *B. clausii*, some *B. cereus*, *B. coagulans*, *B. pumilus*, and *B. licheniformis* have being reported for probiotic potentials.

Bacillus subtilis is a widespread microorganism in nature, which can grow efficiently in low-cost carbon and nitrogen sources [8].

This organism had been reported for antimicrobial, antiviral, and anticancer effects and can be used as a single or mixed type commercial probiotics. Jeon *et al*. [9] reported *B. subtilis* P223 to have inhibited the adhesion of *Salmonella enteritidis*, *Listeria monocytogenes* and *Escherichia coli* to the HT-29 cells. Daneshazari *et al.* [10] revealed probiotic characteristics assessment, especially tolerance to bile salts and acidic conditions of *B. subtilis* strain. The *In vitro* tests for this strain showed similar results to other probiotic strains and could be considered as a probiotic.

Guo et al. [11] reported the inhibitory activity of the spore-forming *Bacillus subtilis* MA139 against *Escherichia coli* K88 and K99, *Salmonella typhimurium* and *Staphylococcus aureus* using a disc plate diffusion assay. The strain showed full resistance to pH 2, 0.3% bile salts, exhibit the highest antimicrobial activity. The strain was selected as a potential probiotic and fed to piglets at concentrations of 2.2 x 10(5), 2.2 x 10(6) or 2.2 x 10(7) CFU/g of feed during a 28-day feeding trial. *Bacillus* spp. were isolated from the digestive tract of freshwater fish, were also assessed for their probiotic potential. Based on the preliminary screening only the *Bacillus* strains FS1, FC3 and FC6 were selected by using *Bacillus* selective media (Himedia) for their probiotics characterization. The biosafety assay confirmed that the isolates were not pathogenic to the host fish. They were able to survive in acidic and alkaline conditions, higher tolerance to bile salt, high surface hydrophobicity to solvents, and were found to tolerate in gastric juice [12].

B. subtilis SOM8 displayed the most promising activities against five listed human enteropathogens and was selected for further comprehensive assessment. It exhibited good tolerance when exposed to adverse stressors including acidity, bile salts, simulated gastric fluid (SGF), simulated intestinal fluid (SIF), and heat treatment. Additionally, it possesses host-associated benefits such as antioxidant and bile salt hydrolase (BSH) activity and contains only haemolysin toxin genes but has been proved to display partial haemolysis in the test [13]. AlGburi *et al.* [14] also reported *B. subtilis* KATMIRA1933 to have inhibited the growth of *Streptococcus intermedius* and *Porphyromonasgingivalis,* tolerate up to 0.3% (w/v) bile salts and survived incubation for 4 h in MRS broth at pH 2.0 to 3.0. The probiotic potential *Bacillus subtilis* BSth-5 and BSth-19 isolated from the gut of healthy fish using *in vitro* assays was also reported[15]. In Antimicrobial assays performed by [16] via radial diffusion, agar spot, and co-culture assays using *Bacillus subtilis* (CP9) from desert camel in vitro, significant bactericidal effect against Enterotoxic*E. coli* (ETEC), *Salmonella typhimurium*and Methicillin-resistant *Staphylococcus aureus* (MRSA) were revealed.

Probiotic *Bacillus subtilis* had been applied in many industries including poultry. Mohamed et al. [17] investigated dietary supplementation with *Bacillus subtilis* (*BS)* ATCC19659 on growth performance, biochemical indices, intestinal morphology, and cecum microflora in broiler chicks. They revealed that the organism as feed additive positively affected growth performance, immunity response, and cecal microflora of broilers.

Bacillus species are reported to be a plant growth promoting rhizobacteria (PGPR) as they can significantly enhance plant growth and represent a mutually helpful plant-microbe interaction due to its ability to form spores that can survive in the soil for long period of time under harsh environmental conditions [18]. Hashem *et al.* [18] reported the stimulation of plant growth by *Bacillus subtilis,* as they solubilize soil, enhance nitrogen fixation and produce siderophores that promote its growth and suppresses the growth of pathogens. In a study conducted by [19]**,** *Bacillus subtilis* NRRL B-30408 was utilized as a potential inoculant for crops grown under rainfed conditions in the mountains. Their result revealed the organism as a good plant growth promoter.

II. Materials And Methods

2.1. Confirmatory test for collected indicator organisms

The indicator organisms (*Staphylococcus aureus, Escherichia coli* and *Salmonella* sp.) collected at University of Nigeria Teaching Hospital, (UNTH) Enugu state, Nigeria was carried out as described by [20].

2.2. Source of Test Organism

The assayed organism *Bacillus subtilis* FFOS was previously isolated from fermented castor oil seed (ogiri) and characterized by [21].

2.3. Screening for probiotic potential of *Bacillus subtilis* FFOS

The following test was carried on *Bacillus subtilis* FFOS to determine its probiotic potentials

2.3.1. Antimicrobial activity

The test organism was spotted onto the surface of Nutrient agar plate and incubated at 37°C for 24h.Therefore 0.1ml of 24h culture indicator strain *(Escherichia coli, Salmonella* sp. and *Staphylococcus aureus)* were inoculated into 7ml of soft brain heart infusion medium (containing 0.7% agar) and carefully layered over the plate containing grown test isolate. The plates were observed for zone of inhibition sand were measured using a meter rule in milliliter (mm)[22].

2.3.2. Bile resistance assay

The ability of the bacterial isolate to resist bile salt was determined using the method of [22].The isolate was inoculated into nutrient agar containing 0.1-0.7% of bile salt and incubated at 37°C for 24h.The culture without bile salt served as the control.

2.3.3. Hemolytic activity assay

The organism was spotted onto blood agar plate and incubated at 37°C for 24h.Thereafter, the plate was observed for formation of any clear zone [22].

2.3.4. Acid toleranceassay

The organism was inoculated (1%v/v) into nutrient broth adjusted to pH 3(acidic condition) and pH7.0 (control) and incubated for 3h at 37°C. After incubation100µl of the sample was spread plated on nutrient agar and incubated at 37°C for 24 h. Thereafter, the developed colonies were counted using a colony counter and enumerated as colony forming unit/ml.

2.4. APPLICATION OF THE TEST ORGANISM IN RELEVANT INDUSTRY

The assayed organism, *Bacillus subtilis* FFOS was applied in the following Areas;

2.4.1. A. POULTRY

2.4.1.1. Source of chicken and its feed

A total number of six, a-day old broiler chickens and the feeds were purchased from Garikki market Enugu state, Nigeria.

2.4.1.2. Preparation of the chicken house

The chicken house was constructed using a wire gauze, partitioned with wood and was roofed with aluminum corrugated sheets. The constructed house was fumigated with insecticide (0.04% permethrin and 0.02% beta -cypermethrin).

2.4.1.3. Feeding Process

Nutrient broth (3%) containing the test organism was supplemented in the chicken feed (chicken marked with red ink). The feed without supplement was used to feed the control chickens (without mark). The chicken feeding process was carried out for 12weeks and the growth rate of each chicken determined every week using a weighing balance (Medifield Equipment and Scientific Ltd,England).

2.4.2. B. FARMING

2.4.2.1. Planting Process

Loamy soil collected from Agricultural garden in Enugu State University of Science and Technology, (ESUT) Nigeria was dispensed into three transparent bottles respectively. Thereafter, 2-bean seeds were planted into the bottles respectively. Plant in bottle 1 (Sample A) was grown with nutrient broth containing the test organism, *Bacillus subtilis* FFOS; plant in bottle 2 (Sample B) was grown with nutrient broth alone and bottle 3(Sample C) was grown with water alone. Their growth rates were monitored daily until 12days.

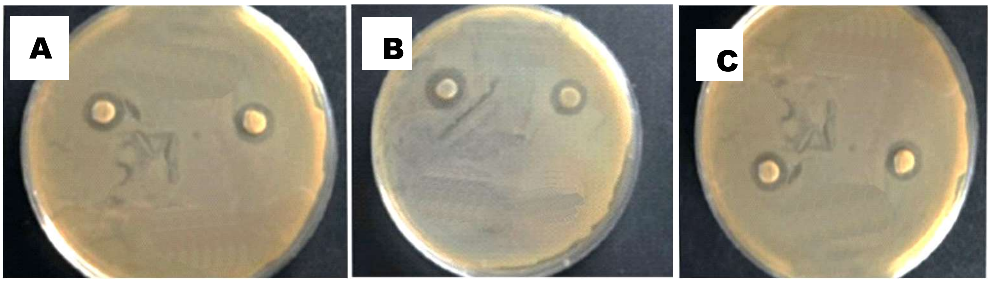
2.5. STATISICAL ANALYSIS

The obtained data from the samples were carefully analyzed and compared using statistical package analysis of variance (ANOVA) .

III. Results And Discussion

3.1. Antimicrobial activity assay

Figure 1 revealed the antimicrobial activity of *Bacllus subtilis* FFOS on test pathogens. The assayed organism had antimicrobial potential against all the test pathogens. The antimicrobial ability was more with *Escherichia coli* (8-10mm). The obtained result is similar with the result of [16] that reported *Bacillus subtilis* (CP9) with significant bactericidal effect against Enterotoxic *E. coli* (ETEC), *Salmonella typhimurium* and Methicillin-resistant *Staphylococcus aureus* (MRSA).



Legend: A= Staphylococcus aureus(7-9mm), B= Escherichia coli (8-10mm), C= Salmonella sp. (6-9mm)

Figure 1: Antimicrobial activity of *Baclus subtilis* FFOS on test pathogens

3.2. Bile resistance assay

Bacillus subtilis FFOS (Table 1) was able to withstand bile up to 7% concentration (4.0 x10^4CFU/mL). There was no significant difference in all the assayed bile concentration when compared to that of the control samples ($p<0.05$). The obtained result is in line with the findings of [11] that stated that *Bacillus subtilis* is known to exhibit good tolerance when exposed to adverse stressors including acidity, bile salts, simulated gastric fluid (SGF), simulated intestinal fluid (SIF), and heat treatment. The result is similar with the results of [14] that recorded same result with *B. subtilis* KATMIRA1933.

Table1: Bile Resistance Assay of *Bacillus subtilis* FFOS

	Microbial Growth (CFU/mL)	
Bile Concentration (%)	*Bacillus subtilis* FFOS	Control sample
1	4.5x10^4	4.6 x10^4
2	4.3 x10^4	4.3 x10^4
3	4.0 x10^4	4.1 x10^4
4	4.0 x`10^4	4.0 x10^4
5	4.1 x10^4	4.2 x10^4
6	3.9 x10^4	4.1 x10^4
7	4.0 x10^4	4.1 x10^4

3.3. Hemolyticactivity assay

The hemolytic activity revealed gamma hemolysis as no haemolysis was detected (Figure 2). This feature of *Bacillus subtilis* FFOS shows that the organism when administered as probiotics will not affect the red cell hemoglobin. The obtained result is similar with that of [23] that reported non hemolytic activity of *Bacillus subtilis* LR1 isolated from the gastrointestinal tract of Labeorohita. However [25] recorded partial hemolysis of *Bacillus subtilis* (PTCC 1023)

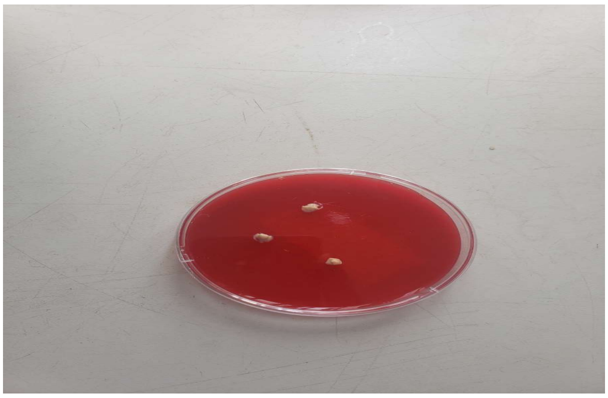


Figure 2: Hemolytic activity of *Bacillus subtilis* FFOS

3.4. Acid toleranceassay

Figure 3 indicated the acid tolerance assay. The assayed organism was able to withstand acidic conditions (pH 3) up to 97.5 %. The ability of *Bacillus subtilis* FFOS to withstand acidic conditions pH3 indicated that it will survive in the acidic gastric environment in the stomach when used as a probiotic.

Similar result was revealed by Ritter et al. (2018) that recorded more than 90% acidic condition (pH 2) survival of *Bacillus subtilis* FTC01 and CP01. Lee *et al.* [4] recorded *Bacillus subtilis* JSP1 with full resistant to both pH 2 and 3, whereas *Bacillus subtilis* SM2 showed relatively good viability at pH 3.

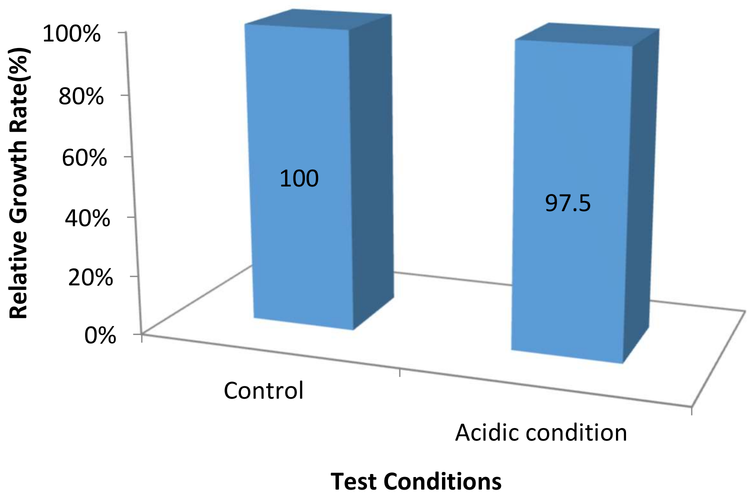


Figure 3: Acid toleranceassay of *Bacillus subtilis* FFOS

3.5. APPLICATION IN RELEVANCE INDUSTRY

3.5.1. A. POULTRY

The effect of *Bacillus subtilis* FFOS culture on chicken growth was stipulated in Figure 4. *Bacillus subtilis* FFOS significaantly enhanced the chicken growth (4.01kg/12wks)($p<0.05$). The findings agreed with the statement of [17] that stated that *Bacillis subtilis* when used as feed additive positively affected growth performance, immunity response, and cecal microflora of broilers. Mohamed *et al.* [17] investigated dietary supplementation with *Bacillus subtilis* (*BS)* ATCC19659 on chicken (broiler) and recorded a significant growth.

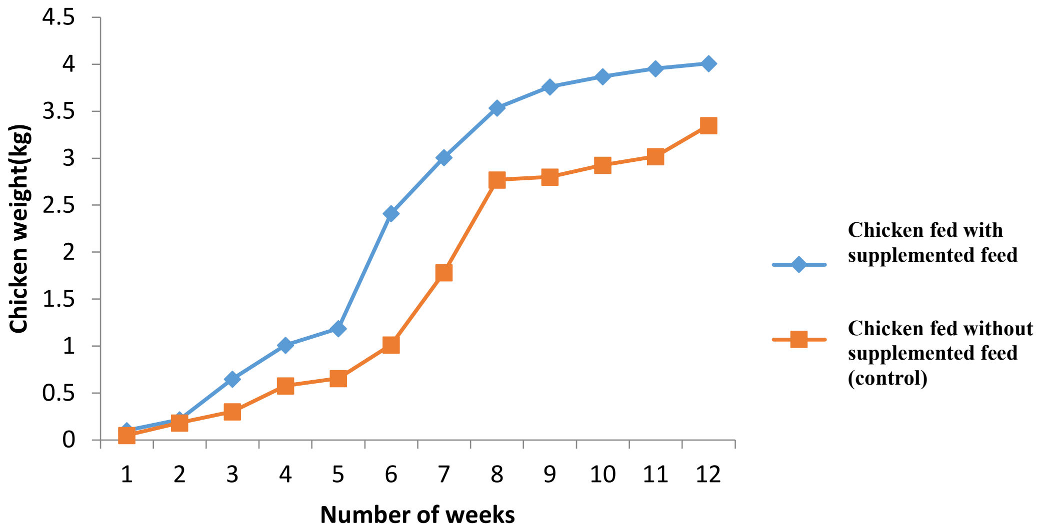


Figure 4: Effect of *Bacillus subtilis* FFOS culture on chicken growth

Chicken fed with supplemented feed(4.01kg/12wks)

Chicken fed without supplemented feed(control: 3.35kg/12wks)

Figure 5: Image of test chickens (12wks)

3.5.2. B. FARMING

The image of plant growth of the assayed samples was stipulated in Figure 6. The broth supplemented with *Bacillus subtilis* FFOS enhanced the plant growth more than that without supplement and the one grown with water alone. The plant grown with water alone has higher growth than that grown with broth alone. The result is in agreement with [18] that stated that *Bacillus subtilis* stimulates plant growthas they solubilize soil, enhance nitrogen fixation and produce siderophores that promote plant growth. The obtained result similar with [19] that also reported significant increase in plant growth with addition of *Bacillus subtilis* NRRL B-30408 during the planting process,

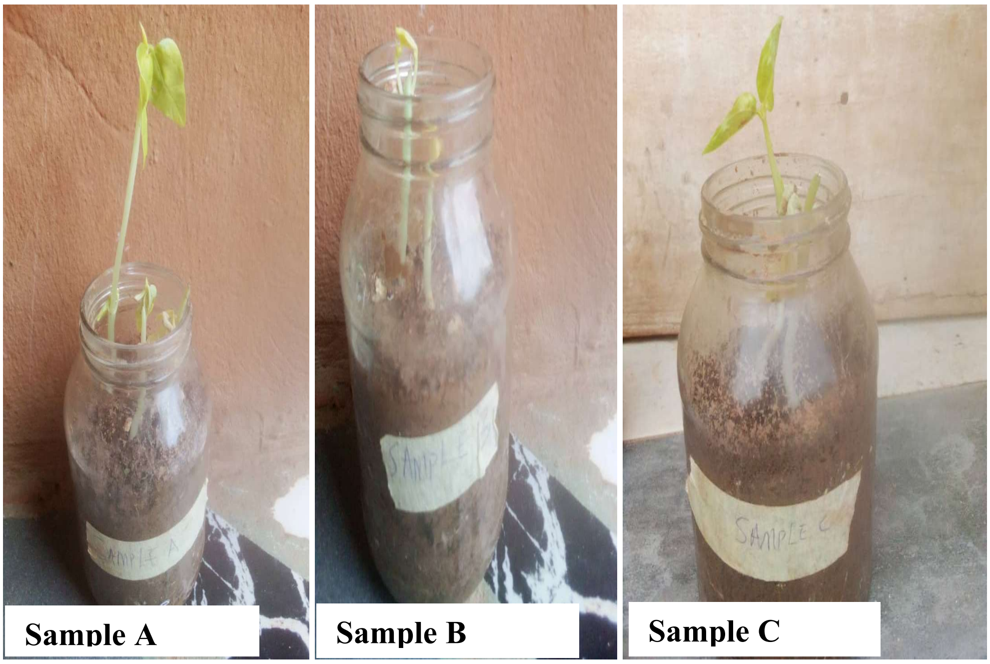


Figure 6: Image of plant growth of the assayed samples

Legend:Sample A=Plant grown with Nutrient broth +*Bacillus subtilis* FFOS; Sample B= Plant grown with Nutrient broth alone; Sample C= Plant grown with water alone

IV. CONCLUSION

The obtained result revealed *Bacillus subtilis* FFOS; a probiotics, good chicken growth enhancer and plant growth promoter. Therefore, this organism can be used as probiotics and can be applied in agriculture as chicken and plant growth promoter.

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