ACTA SCIENTIFIC MICROBIOLOGY (ISSN: 2581-3226)

Volume 2 Issue 7 July 2019

Research Article

Antimicrobial Activity of Bacillus Subtilis Against Some Selected Food Borne Pathogens

Temilade Praise Ozabor[1]* and Ilesanmi Festus Fadahunsi[2]

[1]*Department of Microbiology, Osun State University, Osogbo, Nigeria*

[2]*Department of Microbiology, University of Ibadan, Oyo State, Nigeria*

***Corresponding Author:** Temilade Praise Ozabor, Department of Microbiology, Osun State University, Osogbo, Nigeria.

Received: May 13, 2019; **Published:** June 17, 2019

DOI: 10.31080/ASMI.2019.02.0278

Abstract

The use of synthetic chemicals in food preservation have constituted threats to consumers due to their apparent hazardous side effects. Therefore, there is need to search for effective alternative with minimal side effects. An attempt is made in this study to evaluate the antimicrobial activity of Bacillus subtilis metabolites against selected food borne pathogens with the view of determining its potentials in food preservation and shelf-life extension.

Bacteria were isolated from ogiri, okpehe, and iru. They were identified using culture dependent method and morphological and biochemical characterization. The inhibitory substances such as alkaloids, lactic acid, saponin and enzymes present in the filterate of the bacteria were screened for using standard methods while antimicrobial activity was determined using the agar well dilution method. The minimum inhibitory concentration and the minimum bactericidal concentration was estimated using the broth tube dilution method.

Isolates were identified as *Bacillus subtilis, Bacillus licheniformis, Bacillus pumilus* and *Bacillus polymyxa*. However, *B. subtilis* showed the highest antimicrobial activity and was used for further studies. The screening experiments showed alkaloids, lactic acid, saponin and enzymes were present in the filtrate. *B. subtilis* metabolites inhibited: *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae, Salmonella typhi, Bacillus cereus, Corynebacterium diphtheria* and *Shigella* dysentriae showing inhibitory values of 15mm, 10mm, 11mm, 8mm, 4mm, 12mm, 7mm, 10mm and 12mm respectively which were significantly different at p±≤0.05. Therefore, it can be concluded that *B. subtilis* metabolites demonstrated high inhibitory zones and could be used as bio preservative agent in foods.

Keywords: *Bacillus subtilis;* Minimum Inhibitory Concentration; Minimum Bactericidal Concentration; Optimization; Agar Well Diffusion

Introduction

The genus *Bacillus* are a group of Gram-positive aerobic endospore forming bacteria that are rod shaped. They are ubiquitous in nature, produced among genus *Streptomyces* and *Bacillus* [1]. A very vital functional property attributed to the *Bacillus* strains is the ability to produce different types of antimicrobial compounds having a broad spectrum of activity against bacteria and fungi [2]. The incidence of infections have been drastically increased over the past three decades, and this trend is likely to continue because some microorganisms have continue to develop multi-drug resistance against antibiotics treatment, therefore, there is need for alternative sources of antibiotics from natural products such as food [3]. Bacillus species have been reported in literature to produce natural bioactive compounds that possess diverse forms of structures and showing a broad spectrum of biological activities against food borne pathogens [3].

The *Bacillus* species are able to produce different types of metabolites that are stable over a wide range of pH and temperature. They secrete numerous types of extracellular carbohydrases such as amylases, glucanases, cellulases, pectinases and pullulanases [2].

The increase of antibiotic resistant bacteria in food during the past few decades and the consumer demand for foods without or with a reduced use of chemical preservatives has geared up the search for natural antimicrobial agents such as foods, plant extracts, enzymes, bacteriopages, essential oils and antimicrobial peptides that are ideal for food preservation due to the absence of harmful side effects on humans and their broad spectrum of activity against specific food borne pathogens Nasser and Samar [4].

To produce these metabolites from Bacillus species, it is important to design an appropriate fermentation medium. Developing a suitable and an ideal basal medium will play an important role in the metabolite optimization [5]. secondary metabolites production is influenced by various environmental factors including nutrients such as: nitrogen, phosphorus and carbon sources, growth rate, oxygen supply, light, temperature, etc. [6-8]. In addition, production of valuable metabolites by microbes differs qualitatively and quantitatively depending on the strains used in fermentation.

The production of antimicrobial metabolites, enzymes, lactic acid, saponin and alkaloid have shown antagonistic activity against various Gram positive and Gram negative bacteria, Ouoba., et al. [9], Xie., *et al* [10]. According to literature, more than 500 biopreservatives have been discovered and the genus Bacillus have produced more than 50 antimicrobial compounds some of which are used as pharmacological agents, food spoilage control agents while others are used to control plant diseases, Stein [11]. According to Odunfa (1985), food condiments such as iru, dawadawa, ogiri, okpehe and ugba constitutes 10% of the daily calorie intake and 5% of the total protein intake. They are produced by spontaneous fermentation in the homes by using rudimentary utensils. Studies have shown that aerobic spore-forming bacteria, especially the Bacillus species are the dominant microorganisms in their fermentation and that due to the presence of proteolytic activities, the bacteria are able to release amino acids, ammonia and other volatile compounds which helps to give pleasant aroma, taste and texture to the food products.

This study was therefore carried out to screen for antibacterial activities of Bacillus subtilis from fermented food condiments against selected food spoilage pathogens with the aim of using it as a bio-preservative agent.

Materials and Methods

- **Sample collection:** Fresh ogiri, okpehe, iru woro and iru pete samples were purchased randomly from local markets in Osogbo and Ibadan metropolis and transported in sterile polythene bags to the University of Ibadan Food and Biotechnology Laboratory for further works.

- **Isolation of bacteria:** Isolation of *B. subtilis* from ogiri, okpehe, iru woro and iru pete was carried out by employing serial dilution using the method of Oguntoyinbo., *et al* [12]. 10g of the samples was weighed and serially diluted to a dilution of 10^{-4} and 10^{-5}. 0.5ml of the diluents were plated out using the pour plate method on 20ml nutrient agar and incubated aerobically for 24-48 hr at 30°C and the plates was examined for microbial growth.

- **Identification of bacteria:** The plates were examined for microbial growth and pure cultures obtained was maintained on nutrient agar slants in MacCartney bottles and stored in the refrigerator at 4°C. Pure isolates were identified based on morphological and biochemical characteristics such as indole, nitrate, citrate and urease with reference to Bergy's manual of systematic bacteriology [12].

Determination of inhibitory substances produced by *B. subtilis*

Alkaloids

One gram of the sample (W) was transferred into an conical flask containing 20 ml 10% acetic acid and ethanol. The solution was shaken, allowed to stand for 4 hours and filtered. The filtrate was evaporated to about a quarter of its original volume and one drop of concentrated ammonia was added. The precipitate formed was filtered through a weighed (W1) filter paper and left to dry in the oven at 600 C. The dried filter paper was weighed to a constant weight (W2) and the % alkaloids was calculated by the formula; %

$$\text{Alkaloids} = \frac{W2 - W1}{W} \times 100.$$ Harborne, 1973.

Saponins

One gram of the sample was transferred into 5 ml 20% ethanol inside a conical flask and placed in a water bath at 550C for 4 hr. The filtered residue was washed with 20% ethanol twice. The extract was reduced to about 5 ml in the oven, and 5 ml of petroleum ether was added. The petroleum ether layer was discarded and 3

ml of butanol was added. The mixture was washed with 5 ml 5% sodium chloride and the butanol layer poured into a weighed petri dish then placed in the oven to evaporate to dryness. The final residue was weighed. Ejikeme., *et al* [13].

Lactic acid

Three drops of phenolphthalin indicator was added to 25ml of B. subtilis supernatant in 150ml Erlenmeyer flask and titrated with 0.1m NaOH until the color changes to pink. One (1) ml of 0.1M NaOH solution is equivalent to 90.80g of lactic acid. Marcela., *et al.* 2016.

Enzyme assay

Cellulase activity

B. subtilis was grown on carboxymethyl cellulose liquid medium (CMC: Peptone, 1.5 g; Yeast extract, 1.5 g, CMC, 35 g; Distilled water, 1 L) at 30°C for 48 hr. The culture was centrifuged at 10,000 g for 20 minutes and the pH adjusted to 7.0. The CMC agar plate was incubated at 37°C for five days to allow for the secretion of cellulose enzyme. At the end of the incubation, the plate was flooded with aqueous solution of Congo red (1% w/v) for 10minutes. The Congo red solution was then poured off and the plate was further treated by flooding with 1 ml NaCl for 10 minutes. The formation of a clear zone of hydrolysis indicated cellulose degradation [14].

Protease activity

B. subtilis was inoculated into liquid Luria medium (Peptone, 10 g; Yeast extract, 5 g; NaCl, 5 g; glucose, 1 g; distilled water, 100 ml) and incubated at 37°C for 24hr. The culture was then filtered to obtain cell free supernatants which were spotted (15 µl on a circle of Whatmann paper of 5 mm diameter) on protease test medium plate. The plates were observed after 24 hr of incubation at 37°C for formation of clear zone of hydrolysis [14].

Antibacterial activity

B. subtilis metabolites was evaluated using the agar well diffusion method. *P. aeruginosa, S. aureus, E. coli, P. mirabilis, K. pneumoniae, S. typhi, B. cereus, C. diphtheriae* and *S. dysentriae* were the indicator organisms used. They were obtained from The Nigerian Institute of Medical Research (NIMR), Yaba, Lagos and grown in nutrient broth, incubated at 37°C for 24 hr. The number of colony forming units (CFU) was determined by plating of dilution 105 of the culture broth onto nutrient agar.

100µl of the cell suspension containing 1.5×108cfu/ml was used to seed the surface of Mueller Hinton agar (MHA) plates by using a sterile swab stick for each plate. Wells of 5mm diameter was cut aseptically into the seeded MHA plates by using a sterile cork borer. A droplet of the MHA was added to each well to prevent leakage. 100µl of the indicator organisms was dispensed into the wells with the aid of a micro liter pipette and allowed to diffuse at room temperature for 18-24 hr. The antimicrobial effects were recorded by measuring the zone of inhibition around the wells. Oyedele and Ogunbanwo [15].

Determination of MIC and MBC

Minimum inhibitory concentration (MIC) was determined by using the "broth dilution technique". A two fold dilution of the cell suspension was used to obtain concentrations of 25mg/ml, 50mg/ml, 100mg/ml respectively. This was done to reduce the concentration of the culture filtrate by half. Two (2ml) of nutrient broth was dispensed into each test tube and sterilized. 1% of the filtrate was introduced into the first and second test tubes, the second test tube was serially diluted. Each of the test tube was inoculated with 100 µl of the indicator organisms containing 1.5×10^8 cfu/ml (0.5 McFarland,s standard) from a 24 hr old culture and incubated at 37ºC for 24h. A tube containing nutrient broth only was differently inoculated with each of the test bacteria and incubated at the same temperature and time, this serves as the control. The tubes were examined for bacterial growth based on turbidity. The minimum inhibitory concentration is the lowest concentration of *B. subtilis* metabolites that completely inhibits the growth of the indicator organisms.

To determine the MBC, one ml of broth was collected from the tubes which did not show any growth and inoculated on sterile nutrient agar by streaking. After incubation the concentration at which there was no visible growth was recorded as the minimum bactericidal concentration [16]. MBC is defined as the lowest of concentration of *B. subtilis* at which 99.9% of the indicator organisms were killed. Each experiment was repeated twice. Turbidity or cloudiness was observed in the broth culture and bacteria growth colonies were observed on the nutrient agar plates [16].

Results

Results of alkaloid, saponin and lactic acid showed that the *B. subtilis* metabolites contained moderate quantities of alkaloids and saponin and abundant quantity of lactic acid.

The antimicrobial activity of *B. subtilis* was not significantly different in *K. pneumoniae* and *Proteus mirabilis.* Showing inhibition zones of 4.00 ± 1.5 and 8.00 ± 0.58 respectively at 100mg/ml. The

highest activity was observed in *Pseudomonas aeruginosa*, having an inhibition zone of 15.00 ± 1.53 at 100mg/ml. Hence, the metabolites of B. subtilis showed highest antimicrobial activity against *Pseudomonas aeruginosa*.

S/no	Tests	Probable organism (Bacillus subtilis)
1	Starch hydrolysis	+
2	Citrate	+
3	Gelatin hydrolysis	+
4	Glucose	+
5	Mannitol	+
6	Maltose	+
7	Sucrose	+
8	Galactose	+
9	Fructose	+
10	Lactose	+
11	Mannose	-
12	Xylose	+
13	Casein hydrolysis	+
14	Indole	-
15	Methyl red	+
16	Voges Proskauer	+
17	Urease	+
18	Growth in 5% NaCl	+
19	Growth in 10% NaCl	+
20	Nitrate	+

Table 1: Biochemical characteristics *B. subtilis* isolated from ogiri, iru and okpehe.

Legend: "+" (positive); "-" (negative)

Constituents	Results
Alkaloids	++
Saponin	++
Lactic acid	+++

Table 2: Qualitative determination of inhibitory substances: alkaloids, saponin, lactic acid and enzymes respectively

Key: ++ moderately present, +++ abundant

Test Organisms	Diameter of inhibition in mm (Mean ± S.D) at 100mg/ml
Klebsiella pneumoniae	4.00 ± 1.5^{a}
Corynebacterium diphtheriae	10.00 ± 1.41^{c}
Shigella dysentriae	12.00 ± 0.00^{d}
Bacillus cereus	7.00 ± 0.70^{b}
Escherichia coli	11.00 ± 0.00^{d}
Pseudomonas aeruginosa	15 .00± 1.53^{e}
Proteus mirabilis	8.00 ± 0.58^{ab}
Staphylococcus aureus *Salmonella typhi*	10.00 ± 0.00^{b} 12.00± 0.00^{b}

Table 3: Antimicrobial activity of *B. subtilis* metabolites against food spoilage microorganisms.

Means with same superscript letter in each column are not significantly different at p≤0.05

Concentration (mg/ml)				
Indicator organisms	**100**	**75**	**50**	**MIC (mg/ml)**
Klebsiella pneumoniae	-	+	+	100
Escherichia coli	-	-	+	75
Staphylococcus aureus	-	-	+	75
Shigella dysentriae	-	-	+	75
Corynebacterium diphtheriae	-	+	+	100
Bacillus cereus	-	-	+	75
Proteus mirabilis	-	+	+	100
Pseudomonas aeruginosa	-	-	+	75

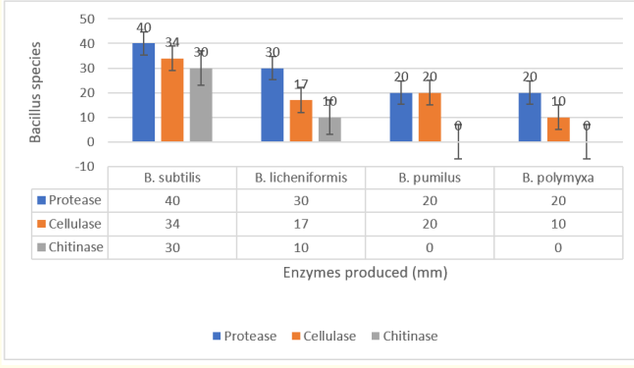
Table 4: MIC of *B. subtilis* metabolites against indicator organisms.

Legend: - (no growth observed); + (growth observed)

Concentration (mg/ml)				
Indicator organisms	**100**	**75**	**50**	**MBC (mg/ml)**
Klebsiella pneumoniae	-	+	+	100
Escherichia coli	-	+	+	100
Staphylococcus aureus	-	+	+	100
Shigella dysentriae	-	+	+	100
Corynebacterium diphtheriae	-	+	+	100
Bacillus cereus	-	+	+	100
Proteus mirabilis	-	+	+	100
Pseudomonas aeruginosa	-	+	+	100

Table 5: MBC of *B. subtilis* metabolites against indicator organisms.

Legend: - (no growth observed); + (growth observed)



	B. subtilis	B. licheniformis	B. pumilus	B. polymyxa
Protease	40	30	20	20
Cellulase	34	17	20	10
Chitinase	30	10	0	0

Figure 1: Enzymatic assay of *Bacillus* species isolated from the fermented condiments.

Discussion and Conclusion

In this study, the predominant organisms isolated from the condiments were *Bacillus* species namely: *B. subtilis, B. licheniformis, B. pumilus* and *B. polymyxa*. The species with the highest frequency of occurrence was identified as *B. subtilis*. This confirms the earlier works reported on the dominance of *Bacillus* species especially *Bacillus subtilis* during the production of indigenous condiments [17], Okanlawon., *et al*. (2010) and Oguntoyinbo., *et al.* [12]. However, reports from Azokpota., *et al*. [18] shows that *Bacillus licheniformis* was the dominant *Bacillus* species isolated in okpehe. This agrees with the findings of this work as *Bacillus licheniformis* was isolated from okpehe. Achi [19] also reported that *Bacillus* species have been demonstrated in other fermenting legume proteins. Bacillus species isolated from variable sources have been reported to be proteolytic and are able to break down oils [9]. From this research work, *B. subtilis* had the highest level of occurrence (49%), *B. licheniformis* (23%), *B. pumilus* (17%) and *B. polymyxa* (11%). *Bacillus* species have been reported to produce antimicrobial inhibitory substances against various Gram positive and Gram negative bacteria, Perez., *et al.* (1992), Ouoba., *et al* [9], Xie., *et al*. [10], Demirkan and Usta, (2013). They also worked on the antimicrobial activities of *Bacillus* species. In support of this work, the antimicrobial activities of *Bacillus subtilis* have been documented by the earlier works of Zheng and Slavik [20], Aslim., *et al* [21] and Fernandes., *et al* [22] against different bacteria. The study of Ogbadu., *et al.* [23] shows that various *Bacillus* species were responsible for the fermentation of African locust bean seeds. Previous studies have shown that the main microorganisms involved in the fermentation of castor oil bean into ogiri are *Bacillus* especially *Bacillus subtilis* [24]. According to this study, *B. subtilis* was more dominant in ogiri, *B. licheniformis* in okpehe, *B. pumilus* in irupete and *B. polymyxa* in iruworo.

The increase in pH in alkaline fermentation might be due to the proteolytic activity of the *Bacillus* species responsible for the fermentation [9]. Mohan and Janardhanan [25] reported that the prohibitive cost of animal proteins in developing countries including Nigeria, calls for extensive exploitation of plant protein sources, which are economically cheaper. Ojinnaka., *et al.* [26] reported that there is likelihood of obtaining species that would serve as biotechnologically useful materials as starter cultures for use as biopreservatives of food condiments. Most authors now agree that there is predominant development of Bacillus species during the various legume fermentation processes [9]. Omafuvbe., *et al.* [27] tested three Bacillus species namely: *B. subtilis, B. licheniformis* and *B. pumilus* singly and in combination for their ability to ferment soybean for the production of daddawa. *B. subtilis* as single or member of a mixed starter produced by soy-daddawa, which was considered most suitable as it gave acceptable pure culture condiment supposedly due to its proteolytic enzyme activity and high level of free amino acids. *Bacillus* species have been reported as producers of certain enzymes such as protease, cellulase, amylase, galactanase, glucosidase and fructofuranosidase which are involved in the degradation of carbonhydrates, Omafuvbe., *et al* [27] and Kiers., *et al* [28]. Maatalah., *et al* [29] reported the presence of saponin and alkaloids in *B. species* which are able to show antibacterial activity against most Gram positive bacteria and a few Gram negatives. The presence of lactic acid bacteria in food enhances digestibility, improves nutritional value, therapeutic benefits and also provides safety against pathogens [30-38].

Bibliography

1. Arasu MV., *et al.* "Antibacterial and antifungal activities of polyketide metabolite from marine Streptomyces sp AP-123 and its cytotoxic effect". *Chemosphere* 90.2 (2013): 479-487.

2. Baruzzi F., *et al*. "Antimicrobial compounds produced by Bacillus species and applications in food". *Communicating Current Research and Technological Advances* (2011).

3. Youcef-Ali M., *et al.* "Antifungal activity and bioactive compounds produced by Bacillus mojavensis and Bacillus subtilis". *African Journal of Microbiology Research* 8.6 (2014): 476-484.

4. Nasser E and Samar S Q. "Antimicrobial activity of Bacillus cereus: isolation, identification and the effect of carbon and nitrogen sources on its antagonistic activity". *Journal of Microbiology* 8.2 (2015): 7-13.

5. Jia B., *et al.* "Medium Optimization Based on Statistical Methodologies for pristinamycins production by Streptomyces pristinaespiralis". *Applied Biochemistry Biotechnology* 144.2 (2008): 133-143.

6. Lin J., *et al.* "Effect of Ammonium in Medium on Ansamitocin P-3 production by Actinosynnema pretiosum". *Biotechnology Bioprocess Engineering* 15.1 (2010): 119-125.

7. Ruiz B., *et al.* "Production of microbial secondary metabolites: Regulation by the carbon source". *Critical Review of Microbiology* 36.2 (2010): 146-167.

8. Sánchez S., *et al.* "Carbon source regulation of antibiotic production". *Journal of Antibiotics* 63.8 (2010): 442-459.

9. Ouoba L I I., *et al.* "Volatile compounds of soumbala, a fermented African locust bean (Parkia biglobosa) food condiment". *Journal of Applied Microbiology* 99.6 (2005): 1413-1421.

10. Xie J., *et al.* "Isolation and characterization of a bacteriocin produced by an isolated Bacillus subtilis LFB112 that exhibits antimicrobial activity against domestic animal pathogens". *African Journal of Biotechnology* 8.20 (2009): 5611-5619.

11. Stein T. "Bacillus subtilis.Antibiotics: structures, syntheses and specific functions". *Molecular Microbiology* 56.4 (2005): 845-857.

12. Oguntoyinbo F M., *et al.* "Diversity of Bacillus species isolated from okpehe, a traditional fermented soup condiment from Nigeria". *Journal of Food Protection* 73.5 (2010): 870-878.

13. Ejikeme C M., *et al.* "Determination of physical and phytochemical constituents of some timbers indigenous to Niger Delta Area of Nigeria". *European Scientific Journal* 10.18 (2014): 247-270.

14. Mounia Y A., *et al.* "Antifungal activity and bioactive compounds produced by Bacillus subtilis and Bacillus mojavensis". *African Journal of Microbiology Research* 8.6 (2014): 476-484.

15. Oyedele A O and Ogunbanwo T S. "Antifungal activities of Bacillus subtilis isolated from some condiments and soil". *African Journal of Microbiology Research* 8.18 (2014): 1841-1849.

16. Doughari J H. "Antibacterial activity of Tamarindus indica Linn. Research Department of Microbiology, Federal University of Technology, Yola; Adamawa State, Nigeria". *Tropical Journal of Pharmaceuticals* 5.2 (2006): 597-603.

17. O'Mahony T., *et al.* "The application of a fermented food ingredient containing variacin, a novel antimicrobial produced by Kocuriavarians to control the growth of Bacillus cereus as a powerful biocontrol agent". *Annals of Biochemistry* 54 (2010): 495-508.

18. Azokpota P., *et al.* "Esterase and protease activities of Bacillus species from afitin, iru and sonru, three African locust bean (Parkia biglobossa) condiments from Benin". *African Journal of Biotechnology* 5.3 (2006): 265-272.

19. Achi OK. "Microorganisms associated with natural fermentation of Prosopis Africana seeds for the production of okpiye Plant Foods". *Journal of Human Nutrition* 42.4 (1992): 297-304.

20. Zheng G and Slavik M F. "Isolation, partial purification and characterization of a bacteriocin produced by a newly isolated Bacillus subtilis strain". *Letters of Applied Microbiology* 28.5 (1999): 363-367.

21. Aslim B., *et al*. "Determination of some properties of Bacillus isolated from soil". *Turkish Journal of Biotechnology* 26 (2002): 41-48.

22. Fernandes P V., *et al.* "Antimicrobial activity of surfactants produced by Bacillus subtilis R14 against multidrug resistant bacteria". *Brazilian Journal of Microbiology* 38.4 (2007).

23. Ogbadu L J and Okagbue RN. "Fermentation of African Locust Bean (Parkia biglobosa) Seeds: Involvement of Different Species of Bacillus". *Food Microbiology* 5.4 (1988): 195-199.

24. Ibe U and Orabuike JC. "Production, nutritional, sensory and storage profile of ogiri from castor oil seed". Paper presented at DANIDA Int. Seminar, Ougadougou, Burkina Faso 3: 47-53.

25. Mohan V R and Jonardhanan K. "Chemical analysis and nutritional assessments of lesser known pulses of the genus muccuna". *Food Chemistry* 52.3 (1995): 275-280.

26. Ojinnaka M C., *et al*. "Microbila and enzymatic changes associated with the production of ogiri from castor oil bean using B. subtilis as starter culture". *Sky Journal of Food Science* 2.2 (2013):10-18.

27. Omafuvbe B O., *et al.* "Fermentation of soybean (Glycine max) for soy-daddawa production by starter cultures of Bacillus". *Food Microbiology* 19.6 (2002): 561-566.

28. Kier JL., *et al.* "In vitro digestibility of Bacillus fermented soya bean". *International Journal of Food Microbiology* 60.2-3 (2000): 163-169.

29. Maatalah M B., *et al*. "Antimicrobial activity of the alkaloids and saponin extracts of Anabasis articulate". *Journal of Biotechnology and Pharmaceutical Research* 3.3 (2012): 54-57.

30. Enujiugha V N., *et al.* "Evaluation of Starters for the Fermentation of African Oil Bean (Pentaclethra macrophylla Benth) Seeds". *Nutrition and Food Science* 38.5 (2008): 451-457.

31. Gao H., *et al*. "Medium optimization for the production of avermectin B1a by Streptomyces avermitilis 14-12A using response surface methodology". *Bioresource Technology* 100.17 (2009): 4012-4016.

32. Harborne J B. "Phytochemical methods: A Guide to Modern Techniques of Plant Analysis". Chapman and Hall, London, UK (1973).

33. Huang X., *et al.* "Optimization of sterilization of Salmonella enteritidis in meat by surfactin and iturin using a response surface method". *International Journal of Peptide Response Therapy* 15.1 (2009): 61-67.

34. Kadaikunnan S., *et al.* "In-vitro antibacterial, antifungal, antioxidant and functional properties of Bacillus amyloliquefaciens". *Annals of Clinical Microbiology and Antimicrobials* 14 (2015): 9-15.

35. Liman A A., *et al.* "Lipase Activity in Fermented Oil Seeds of Africa Locust Bean, (Parkia biglobosa),Castor Seeds (Ricinu communis) and African Oil Bean (Pentaclethra macrophylla)". *Nigerian Journal of Basic and Applied Science* 18.1 (2010): 136-140.

36. Marcela P B., *et al*. "Lactic acid production by Lactobacillus rhamnosus B103 from dairy industry waste". *Brazilian Journal of Microbiology* 47.3 (2016): 640-646.

37. Sarkar PK., *et al*. "Bacillus Fermentation of Soybeans". *World Journal of Microbiology and Biotechnology* 9.3 (1993): 295-299.

38. Sirtori LR., *et al.* "Mode of action of antimicrobial peptide P45 on Listeria monocytogenes". *Journal of Basic Microbiology* 48.5 (2008): 393-400.

Volume 2 Issue 7 July 2019