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# Isolation, Characterization and Distribution of *Bacillus* Strains in Some Nigerian Fermented Food and Drinks

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***Abstract*** **– Five samples of fermented foods (Yoghurt, Burukutu, Palmwine, Ogi and garri) were examined for total bacterial count using culture-dependent techniques. The *Bacillus species* isolated were *B. subtilis, B. cereus, B. sphaericus, B. pasteurii, B. thuringiensis, B. coagulans, B. megaterium and B. pumilus.* The incident rate of the *B. subtilis* in the fermented foods was 27.3%. *B. subtilis* was isolated from yoghurt, burukutu, palmwine and garri. *B. sphaericus* occurred in 22.7% of the sample. While *B. cereus* occurred in 18.8% of the fermented food sample, *B. thuringiensis* and *B. coagulans* had incidence rates of 9.09% respectively. However *B. pasteurii, B. megaterium* and *B. pumilus* were each isolated from 4.55% of the food sample. The predominant strain of *Bacillus* isolated was *Bacillus subtilis*. The presence of this microorganisms identified could be harmful to man and pose a serious public health hazard to the consumers of the fermented foods.**

***Keywords*****–Bacillus, Fermented, Foods, Palmwine, Yoghurt.**

## I. INTRODUCTION

Bacteria belonging to the genus *Bacillus* have a long and distinguished history in the realms of biotechnology. They were probably first used by the Japanese in the preparation of a traditional fermented food from rice straw and soyabean, itohiki natto [1]. The bacilli include many versatile bacteria and the most effective bacteria control agents for various insect pests, *Bacillus thuringiensis*. *Bacillus* species are also source of numerous antibiotics, flavor enhancers, surfactants, and various other products [2].

Various traditional fermented foods and conventional foods are produced in many African countries. The common substrates for fermentation are cassava and cereal grains such as maize, sorghum and millet. Fermented foods and drinks in Nigeria include Ogi, burukutu, yoghurt, palmwine, fufu, garri [3]. Fermentation is one of the oldest forms of food preservation technology in the world. Indigenous fermented foods have been prepared and consumed for thousands of years and are strongly linked to culture and tradition especially in rural households and village communities. Locally fermented food is a form of processing where microbes for example, Lactic acid bacteria (LAB) are utilized for production via the process known as fermentation [4]. Fermentation in food processing is the conversion of carbohydrate to alcohol and carbondioxide or organic acids using yeast and or bacterial, under anaerobic conditions [5]. It is one of the classic methods to preserve foods. Lactic acid bacterial (LAB) and yeasts are responsible for most of these fermentations [6], [7], [8].

The primary benefit of fermentation is the conversion of sugars and other carbohydrates, e.g. converting juice into wine, grains into beer, carbohydrate into carbon dioxide to leaven bread, and sugars in vegetables into preservatives organic acids. Fermenting food actually increases the Vitamin in contents and the shelf life [9].

The numerous species of *Bacillus* includes *B.alcalophilus. B.alvei, B.aminovorans, B.atcophacus, B.boroniphilus, B.brevis, B.caldolyticus, B. centrosporus, B. cereus, B. circulans, B. coagulans, B. firmus, B. flawthermus, B. fusiformis, B. globigii, B. infernus, B. larvae, B. latensporus, B. lentus, b. licheniformis, B. megaterium, B. mesentericus, B. mucilaginosus, B. mycoides, B. natto, B. pantotheticus, B. polymyxa, B. pseudoanthracis, B. pumilus, B. schlegalii, B. sphaericus, B. sporethermopphilus, B. subtilis, B. thermoglicosidasius, B. vulgatis, B. welhenstephanensis* [10], [11].

This paper reports the *Bacillus* strains associated with some common Nigerian fermented foods such as Yoghurt, Ogi, Burukutu, Garri, Palm wine.

## II. MATERIALS AND METHODS

Five fermented food samples were collected for this study. The samples examined include; Ogi, Yoghurt, Burukutu, Garri and palm wine. Freshly prepared samples were collected from different preparation points in Ado-Ekiti metropolis. The bacterial load of the fermented food sample was enumerated by plating serially diluted samples on Nutrient agar using the pour plate technique. The samples were also cultured on Hichrome *Bacillus* Agar. Inoculated plates were incubated at 37$^{o}$C for 24 hours. After incubation, distinct colonies were picked and sub cultured into nutrient agar slant for further studies.

The bacterial isolates were identified based on their cultural, morphological and biochemical characteristics. The chromogenic characteristics of the *Bacillus* strains on the Hichrome *Bacillus* were considered in the characterization of the bacterial isolates. The isolates were thus identified with reference to the Robert *et al.* [10] and the Hichrome *Bacillus* agar manual.

## III. RESULTS

In this study, different species of *Bacillus* were from the fermented food samples examined. The total bacterial count on nutrient agar is shown on table 1. The highest count (1.29 x $10^6$ cfu/ml) was observed in yoghurt. While the total *Bacillus* count on Hichrome *Bacillus* agar range from $1.5\pm0.01\times10^5$ cfu/ml to $2.3\pm0.01\times10^5$ cfu/ml. The *Bacillus* species isolated from the fermented foods

samples include *Bacillus subtilis, Bacillus sphaericus, Bacillus thuringiensis, Bacillus cereus, Bacillus pasteurii, Bacillus coagulans, Bacillus megaterium* and *Bacillus pumilus* as shown in table 2. *Bacillus subtilis* had the highest frequency of 27.3%, while *Bacillus pasteurii, Bacillus megaterium, Bacillus pumilus* showed the lowest occurrence of 4.55% each (table 2). *Bacillus subtilis* had the highest occurrence in the entire food sample on Hichrome *Bacillus* agar and nutrient agar (Table 3, 4).

Table 1: Bacterial count of fermented foods (cfu/ml/g)

| Sample | Total bacterial count | Total Bacillus count |
|---|---|---|
| Yoghurt | $1.29\pm0.01\times10^{6}$ | $1.8\pm0.01\times10^{5}$ |
| Burukutu | $9.3\pm0.1\times10^{5}$ | $2.0\pm0.01\times10^{5}$ |
| Palmwine | $7.5\pm0.5\times10^{5}$ | $2.3\pm0.01\times10^{5}$ |
| Ogi (Maize) | $9.5\pm0.3\times10^{5}$ | $1.5\pm0.01\times10^{5}$ |
| Garri | $9.0\pm0.1\times10^{5}$ | $1.8\pm0.01\times10^{5}$ |

Table 2: Distribution of *Bacillus* species in fermented food samples.

| ***Bacillus* species Isolated** | **Fermented foods** | **Number (%)** |
|---|---|---|
| *Bacillus subtilis* | Yoghurt, Burukutu, Palmwine, Garri | 6 (27.27) |
| *Bacillus sphaericus* | Yoghurt, Palmwine, Ogi, Burukutu, | 5 (22.73) |
| *Bacillus cereus* | Burukutu, Palmwine, Ogi, Garri | 4 (18.18) |
| *Bacillus thuringiensis* | Yoghurt, Palmwine. | 2 (9.09) |
| *Bacillus coagulans* | Burukutu, Palmwine | 2(9.09) |
| *Bacillus pasteurii* | Burukutu. | 1(4.55) |
| *Bacillus megaterium* | Ogi | 1(4.55) |
| *Bacillus pumilus* | Garri | 1 (4.55) |
| | **Total** | **22 (100.0)** |

Table 3: *Bacillus* species isolated from fermented foods on Hichrome *Bacillus* Agar

| **Food Samples** | **Names of *Bacillus* species/strains** |
|---|---|
| Yoghurt | *Bacillus subtilis, Bacillus thuringiensis* |
| Burukutu | *Bacillus subtilis, Bacillus cereus, Bacillus coagulans* |
| Palmwine | *Bacillus coagulans, Bacillus cereus, Bacillus subtilis* |
| Ogi | *Bacillus megaterium, Bacillus cereus, Bacillus subtilis* |
| Garri | *Bacillus subtilis, Bacillus cereus, Bacillus pumilus* |

Table 4: *Bacillus* species isolated from fermented foods on Nutrient agar

| **Food samples** | **Names of *Bacillus* species/strains** |
|---|---|
| Yoghurt | *Bacillus subtilis, Bacillus sphaericus, Bacillus thuringiensis* |
| Burukutu | *Bacillus subtilis, Bacillus cereus, Bacillus pasteurii, Bacillus sphaericus, Bacillus coagulans* |
| Palmwine | *Bacillus coagulans, Bacillus subtilis, Bacillus cereus, Bacillus sphaericus, Bacillus thuringiensis* |
| Ogi | *Bacillus sphaericus, Bacillus megaterium, Bacillus cereus, Bacillus subtilis* |
| Garri | *Bacillus subtilis, Bacillus cereus, Bacillus pumilus, Bacillus mycoides* |

## IV. Discussion

The results of the study clearly indicate that all food samples observed contained *Bacillus* species. *Bacillus* species can be obligate aerobes or facultative anaerobes [11]. They include both free living and pathogenic species under stressful environmental conditions the cells produce oral endospores that can stay dormant for extended periods. In this study, three species of *Bacillus* were isolated and identified from yoghurt which had the highest *Bacillus* count. Among the *Bacillus* species encountered *Bacillus subtilis* was predominant.

The Burukutu samples analysed also contained a wide range of the *Bacillus* species namely, *B. subtilis, B. cereus, B. coagulans, B. pasteurii, B. sphericus. Bacillus cereus* and *Bacillus subtilis* are the most encountered in the group. *B. cereus* has been reported to cause food poisoning by the production of an entero-toxin [12, [13]. It was also observed that *B. coagulans, B. cereus, B. subtilis, B. sphaericus* and *B. thuringiensis* were present in the palm wine. The occurrence of these bacterial isolates in palm wine samples however supports the previous reports [14], [15]**.** *Bacillus* species has been discovered to be persisting organisms in palm wine even after 96$^{th}$ hour of collection [16]. These organisms have also been reported to originate from several sources which include tapping equipment, containers, and the environment [17].

*B. cereus, B. subtilis, B. megaterium* and *B. sphaericus* were observed in ogi. The microbiology of ogi and related products during the processing stage up to the finished products has been studied [18], [19]. Among the five *Bacillus* species isolated, *Bacillus megaterium and Bacillus subtilis* are prominent starch degrading bacteria commonly found in Ogi. The Garri sample analyzed in this study contained *B. subtilis, B. cereus and B. pumilus.* One of the notable organisms isolated at the beginning of fermentation of cassava is the *Bacillus* strains [20]. Out of the dominating species was *B. subtilis* and other species found were *Bacillus pumilus*. The sugar fermentation profiles of organisms showed that all are able to ferment different sugars but *Bacillus subtilis* is the major fermenter.

This study has shown that fermented foods contain beneficial and potentially pathogenic microorganisms that could be harmful to man and pose serious public health hazard to the consumers. It is therefore recommended that fermented products should be hygienically and microbiologically safe for human consumption as suggested by Kuhnlein and Receveur [21]. One of the many ways through which this can be achieved is by refining and improving the processes involved in the fermentation.

## References

[1] S. Ueda, *Utilization of soyabean as natto, a traditional Japanese food in molecular biology and industrial application.* eds *Maruo, B., Yoshikawa, H.* (Elsevier, Amsterdam, The Netherlands), 1989, pp. 143-161.

[2] L. M. Prescot, J. P. Harley and D. A. Klein, *Microbiology,* 4$^{th}$ edition, WCB, McGraw Hill, Boston, 1999.

[3] I. A. Adesokan, O. P. Abiola, and M. O. Ogundiya, 'Influence of ginger on sensory properties and shelf life of ogi, a Nigerian traditional fermented food'. *Afr. J. Biotechnol*. 2010, *9(12):* 1803-1808.

[4] P. K. Chelule, H. P. Mbongwa, S. Carries and N. Ggaleni, 'Lactic acid fermentation improves the quality of amahewu, a traditional South African maize based porridge'. *Food Chemistry* 2010, *122(3)*: 656-661.

[5] C. F. William and C. W. Dennis, *Food Microbiology*, fourth edition, McGraw Hill, India: 2011, pp. 330.

[6] A. O. O. Adenike, O. O. Mopelola and A. Ayansina, 'Microbal studies and Biochemical characteristic of control fermented Afryo- a Nigerian fermented food condiment from *Proscopis Africana'*. *Pakistan Journal of Nutrition* 2007, *6(6): 620-627.*

[7] C. O. Adebayo and B. I. Aderiye, 'Antifungal activity of bacteriocins of latic acid bacteria from some Nigeria fermented foods,' *Res. J. Microbial.* 2010, *5:1070-1082.*

[8] R. O. Adeleke and O. A. Abiodun, 'Physiochemical properties of commercial local,' *Pakistan Journal of Nutrition* 2010, *9(9): 853-855.*

[9] A. M. Omemu and O. F. Adeosun, 'Evaluation of hazards and critical control points of Ogi in small processing centers in Abeokuta, Nigeria' *Journal of Applied Biosciences* 2010, *29: 1766-1773.*

[10] S. B. Robert, E. G. D. Murray and R. Nathan, *Bergey's Manual of Determinative Bacteriology*, 7th Ed. USA, Waverly Press Inc. Baltimore 2011.

[11] P. C. B. Turnbull, *Bacillus*: In *Barron's Medical Microbiology* (Barons *et al*., eds) (4th ed.). University of Texas medical Branch, 1996.

[12] C. G. A. Thomas, *Medical Microbiology*, University press, Cambridge, UK, 1994.

[13] V. C. Eze, J. J. Okoye, F. O. Agwung and C. Nnabueke, C.'Chemical and microbiological evaluation of soyabeans Hours bought from local markets in Onitsha, Anambra State, Nigeria' *J. Appl. Sci.* 2008, 3: *39-45.*

[14] M. J. Ikenebomeh and M. O. Omayuli, 'Pathogens survival patterns in palmwine and ogogoro. *Nigeria J. Biotechnol* 1988, *116-129.*

[15] N. Okafor, 'Microbiology of Nigeria palmwine with particular reference to bacteria'. *J. Appl. Bacteriol*, 1975, *33:*81-88.

[16] O. Obire, 'Activity of *Zymomonas* species in palm sap obtained from the areas in Edo state', *Nigeria Journal Applied Science Environmental Management* 2005, *9:25-30.*

[17] S. I. Faparunsi and O. Bassir, 'Microflora of fermenting palm wine,' *J. Food Sci. Technol* 1972, *8:*206-212.

[18] S. A. Odunfa and S. Adeyele, 'Microbiological changes during the traditional production of "Ogi baba" West African fermented sorghum gruel'. *J. Cereal Sci*, 1985, *3:* 173-180.

[19] S. A, Odunfa and O. D. Teniola, 'Microbial assessment and quality of "ogi" during spoilage *World J. Microbiol, Biotechnol*, 2002, *8:* 731-733.

[20] O. B. Oyewole, and S. A. Odunfa, 'Microbiological studies on cassava fermentation for lafun production'. *Food Microbiol*. 1988, *5:* 125-133.

[21] H. V. Kuhnlein and O. Receveur, 'Dietary change and traditional food systems of indigenous people'. *Annual. Rev Nutri*; 1996, *16: 417-442.*