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Article

Biomedicine

Probiotic Potential Of Nigerian Fermented
Food *Pentaclethra macrophylla* (Ugba)Moses Nnaemeka ALO¹, Uchenna Iyioku UGAH², Elkena Chubike Ndie³, Boniface N UKWA⁴,
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ABSTRACT [ENGLISH/ANGLAIS]

Probiotics are beneficial bacteria which play pivotal roles in increasing host resistance to colonization by exogenous, pathogenic organisms. This is achieved through different mechanisms. Most of the probiotic species belong to the genera *Lactobacillus* and *Bifidobacterium*. African oil bean seed (*Pentaclethra macrophylla*) popularly called *Ugba* in the local parlance belong to the leguminous family *mimosa cease*. The seed is allowed to undergo fermentation before consumption; it is consumed as a supplement mixed with other traditional foods and as a whole meal. One hundred and eighty five adult subjects comprising of 20 control and 165 test subjects were recruited for this study. Stool samples were collected on the first day and the baseline average bacterial count was obtained after culture using Lactose broth, Eosine Methylene Blue agar and Desoxycholate citrate agar. The test group subjects were fed with *Ugba* for 14 days and stool samples were collected at two-day interval for a total of 22 days and cultured on the three media. There was an increase in the average bacterial counts from 1.5×10^3 and 1.8×10^3 for *Bifidobacterium spp* and *Lactobacillus spp* respectively to 3.6×10^5 and 3.5×10^5 for both organisms respectively on the 14th day when their diet was terminated, this fell to 1.7×10^5 and 2.5×10^5 for both organisms respectively. There was also a reduction in the number of pathogenic bacteria in the subjects with pathogenic bacteria declining from 91 cfu at baseline to 22 cfu after 7 days of consistent consumption of fermented oil bean seeds. Our study showed that constant intake of *Ugba* triggers an increase in the growth and population of the probiotic bacteria *Bifidobacterium spp* and *Lactobacillus spp*. This increase in growth may enhance a reduction in the population of pathogens by competitive inhibition. The difference in the presence of pathogenic bacteria among the test subjects before and during the diet intake was statistically significant ($p < 0.05$) also, the difference in the average colony counts of *Bifidobacterium spp* and *Lactobacillus spp* at the baseline and on the 14th day of intake of the diet was statistically significant ($p < 0.05$).

Keywords: Ugba, probiotics, *Pentaclethra macrophylla*, oil bean seed

RÉSUMÉ [FRANÇAIS/FRENCH]

Les probiotiques sont des bactéries bénéfiques qui jouent un rôle essentiel dans l'augmentation de la résistance de l'hôte à la colonisation par des organismes pathogènes exogènes. Ce résultat est obtenu grâce à des mécanismes différents. La plupart des espèces probiotiques appartiennent aux genres *Lactobacillus* et *Bifidobacterium*. Semences de haricot de pétrole africain (*Pentaclethra macrophylla*) populairement appelé *ugba* dans le jargon local appartient à la famille des légumineuses *mimosa cesse*. La graine est autorisée à subir la fermentation avant la consommation, il est consommé comme un complément mélangé à d'autres aliments traditionnels et comme tout un repas. Cent quatre vingt cinq sujets adultes comprenant de 20 contrôle et 165 sujets d'essai ont été recrutés pour cette étude. Des échantillons de selles ont été prélevés sur le premier jour et le nombre de bactéries moyenne de référence ont été obtenus après culture en utilisant le bouillon lactose, Eosine Bleu de Méthylène agar agar et le citrate Desoxycholate. Les sujets du groupe d'essai ont été nourris avec *ugba* pendant 14 jours et les échantillons de selles ont été prélevés à des intervalles de deux jours pour un total de 22 jours et on les cultive sur les trois supports. Il y avait une augmentation dans les comptages bactériens moyenne de 1.5×10^3 et 1.8×10^3 pour *Bifidobacterium spp* et *Lactobacillus spp* respectivement à 3.6×10^5 et 3.5×10^5 pour les deux organismes, respectivement le 14^e jour quand leur régime alimentaire a été arrêtée, ce chiffre était tombé à 1.7×10^5 et 2.5×10^5 respectivement pour les deux organismes. Il y avait aussi une réduction du nombre de bactéries pathogènes dans les sujets avec des bactéries pathogènes en baisse de 91 ufc au départ à 22 ufc après 7 jours de consommation constante de fermentation des graines de haricots à l'huile. Notre étude a montré que l'apport constant de *ugba* déclenche une augmentation de la croissance et de la population de bactéries probiotiques *Bifidobacterium spp* et *Lactobacillus spp*. Cette augmentation de la croissance peut améliorer la réduction de la population de pathogènes par inhibition compétitive. La différence dans la présence de bactéries pathogènes chez les sujets de test, avant et pendant la prise d'alimentation était statistiquement significative ($p < 0,05$) également, la différence entre les comptages de colonies moyennes de *Bifidobacterium spp* et *Lactobacillus spp* à la ligne de base et le 14^e jour de l'apport de l'alimentation était statistiquement significative ($p < 0,05$).

Mots-clés: Ugba, les probiotiques, *Pentaclethra macrophylla*, des semences de haricots à l'huile

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INTRODUCTION

Probiotics is a general name for microorganisms that are associated with beneficial effects for humans and animals. They contribute to intestinal microbial balance and play a role in maintaining health (Soccol *et al.*, 2010). Probiotics play a vital role in increasing host resistance to colonization by exogenous, potentially pathogenic organisms. This is achieved through different mechanisms such as production of lactic acid, hydrogen peroxide or acetic acid which increases the acidity of the intestine and inhibits the reproduction of numerous pathogenic bacteria (Reid *et al.*, 2003).

Probiotics has been found in dairy and non – dairy products (Soccol *et al.*, 2010). They are usually consumed after antibiotic therapy which destroys the microbial present in the digestive tract. Regular consumption of food containing probiotic microorganisms is recommended to establish a positive balance of the population of beneficial microbes in the intestinal flora (Soccol *et al.*, 2010).

Most of the probiotic species belong to the genera *Lactobacillus* and *Bifidobacterium* (Blandino *et al.*, 2003; Manning and Gibson, 2004; Commane *et al.*, 2005). Benefits of consumption of probiotics include the prevention and treatment of infantile diarrhoea, colon cancer, constipation, antibiotic induced diarrhoea, hypercholesterolaemia, lactose intolerance, vaginitis and intestinal infections (Marchand and Vandenplas, 2000).

African oil bean seed (*Pentaclethra macrophylla*) popularly called *Ugba* in the local parlance belong to the leguminous family *mimosa cease*. It is frequently cultivated in forest areas. At maturity the pods explode and disperse the seeds. The raw seed is a potential source of edible protein, energy and fatty acids (Enujiugha and Agbede, 2000). The seed is then allowed to undergo fermentation before consumption. *Ugba* is an alkaline – fermented food, the traditional production is done with rudimentary equipment. It is used sometimes as part of a main meal or as an additive. However, it plays an important role in the diet of the populations residing in South Eastern Nigeria.

Probiotic bacteria are essential for healthy gastrointestinal function. The action of probiotics on intestinal flora results in salient benefits to the host such as positive effects on host nutrition and colonic health (Umesaki and Setoyama, 2000) as well as protection against pathogens and development of the immune system (Isolauri *et al.*, 2002). This study aimed at investigating the probiotic potential of fermented *pentaclethra macrophylla* seeds in Ebonyi State, South-

Eastern Nigeria, it also sought to determine if *Ugba* possesses any antibiotic potential.

MATERIALS AND METHODS

Ethical Clearance

Informed consent was obtained from all the subjects. Ethical clearance was obtained from the ethics committee of Federal Teaching Hospital Abakaliki, Ebonyi State where the laboratory analysis was performed.

Exclusion Criteria

Persons who had taken any antibiotic within the last 7days from the commencement of the diet were excluded from this study. Subjects were instructed to abstain from any antibiotic within the duration of the study - those who took antibiotic regimen after commencement of the study were disqualified.

Study Population

One hundred and eighty five adults (comprising of 90males and 95 females) living in Abakaliki metropolis were randomly selected for the study. Among the population, twenty subjects (10males and 10 females) were used as the control groups and did not consume *Ugba* within the duration of the study, while 165 (80 males and 85 females) were the test group and were fed with fermented Oil bean seeds for 14 consecutive days.

Sample Collection

Stool samples were collected from all the subjects into a sterile wide mouthed screw capped containers. The stool samples were collected on the first day before the subjects were placed on the diet and the bacteria isolates were taken as the population at baseline. Subsequently, stool samples were collected at 2 days interval for up to the 20th day from the first day of the diet consumption.

Sample Processing

One gram (1g) of the stool sample was diluted in 9ml of sterile normal saline. Then a serial tenfold dilution was made up to a dilution of 10⁻⁶. From this last dilution, 20µl was dispensed into lactose broth (Lab M Limited, Topley house, 52 Welsh Lane, Lancashire, UK) 20µl was also dispensed onto the surface of Eosime Methylene Blue agar and Desoxycholate Citrate agar (Wade road, Basingstoke, Hampshire, UK) and spread out using a sterilized wire loop. The media were subsequently incubated at 37°C for 24 hours. Bacteria colony count was performed for all the isolates and the average colony

count for all the subjects was obtained, the bacteria isolates were identified and characterized using standard methods. The population of bacteria was estimated by multiplying the colony count with the dilution factor. The average colony count for all the subjects was taken for each batch of samples. Aseptic techniques were strictly adhered to and all standard operating procedures were judiciously followed.

Data Analysis

Data was analyzed using Chi squared test. A $p < 0.05$ was considered significant. Data analysis was done with the aid of Statistical Programme for Social Sciences (SPSS) version 17.0.

RESULTS

The average colony count for all the subjects were taken after identification and characterization of the bacteria isolates. Among the control group, the average colony count did not increase from the baseline count while among the test subjects, the average colony count observed for each day sampled rose above the baseline (Table 1). When the baseline stool sample was cultured (day 0), heavy growth of pathogenic bacteria including *Shigella spp.*, *Salmonella typhi*, *Salmonella paratyphi* and *Proteus mirabilis* were isolated from 39, 27, 16 and 9 subjects respectively. Within seven days from the commencement of the diet, the number of subjects with positive cultures for the pathogenic bacteria reduced to 8, 5, 7 and 2 for the organisms respectively (table 2).

Table 1: This table shows average colony count of probiotic bacteria among the subjects

Day	Average Colony Counts	
	<i>Bifidobacterium spp.</i>	<i>Lactobacillus spp.</i>
0	1.5×10^3	1.8×10^3
2	1.9×10^4	2.3×10^4
4	2.1×10^4	2.5×10^5
6	2.2×10^5	2.6×10^5
8	1.9×10^5	2.8×10^5
10	2.5×10^5	2.9×10^5
12	3.5×10^5	3.3×10^5
14	3.6×10^5	3.5×10^5
16	3.2×10^5	3.2×10^5
18	2.2×10^5	2.6×10^5
20	1.7×10^5	2.5×10^5

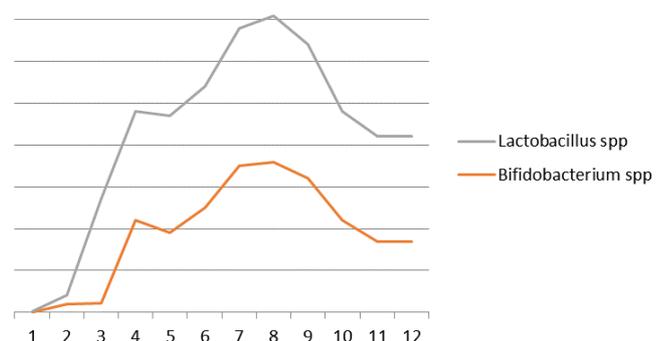
It was observed that among the test subjects, *Bifidobacterium spp* and *Lactobacillus spp* rose from the baseline colony count of 1.5×10^3 and 1.8×10^3 cfu/ml respectively to 3.6×10^5 and 3.5×10^5 cfu/ml respectively on the 14th day when the *Ugba* was withdrawn from the diet of the subjects. Six days after withdrawal from the diet, (on the 22nd day) the average colony counts fell to 1.7×10^5 cfu/ml and 2.5×10^5 cfu/ml for *Bifidobacterium spp* and *Lactobacillus spp* respectively.

The difference in the presence of pathogenic bacteria among the subjects before and during the diet intake was statistically significant ($p < 0.05$). The difference in the average colony counts of *Bifidobacterium spp* and *Lactobacillus spp* at the baseline and on the 14th day of intake of the diet was also statistically significant ($p < 0.05$).

Table 2: This table shows information about pathogens isolated from the subjects at baseline and after 7 days of diet intake

Exam	Number at baseline	Number after 7 days
<i>Shigella spp</i>	39	8
<i>Salmonella typhi</i>	27	5
<i>Salmonella paratyphi</i>	16	7
<i>Proteus mirabilis</i>	9	2
Total	91	22

Figure 1: This figure shows the bacteria growth curve of the average bacteria counts ($\times 10^5$) over the days studied



DISCUSSION

Oil bean seed (*Pentaclethra macrophylla*) called *Ugba* in Nigeria is a local delicacy mostly eaten in the South

Eastern part of the country. It is consumed as a supplement mixed with other traditional foods and as a whole meal.

In our study, we observed an increase in the average colony counts of *Bifidobacterium spp* and *Lactobacillus spp* among the test group whereas there was no significant increase in the average colony count of these bacteria among the control group. This is an indicator that frequent consumption of fermented oil bean seed stimulates the proliferation of these bacteria which are well known probiotic bacteria.

Heavy growth of pathogenic bacteria was isolated from some of the subjects; these include *Shigella spp.*, *Salmonella typhi*, *Salmonella paratyphi* and *Proteus mirabilis* (Table 2). However, following consistent intake of the *Ugba*, there was a sharp decline in the number of subjects with these bacteria after 7 days (from 91 to 22) even in the absence of antimicrobial therapy. This indicates a direct correlation between the increase in the population of the probiotic bacteria and the reduction in the population of pathogenic bacteria.

Different mechanisms have been used to explain the way in which probiotics perform their function. Some lactic acid bacteria involved in fermentation are able to produce hydrogen peroxide, but they do not possess true catalase to break down the hydrogen peroxide. Therefore, the hydrogen peroxide can accumulate and be inhibitory to some harmful bacteria and to the lactic acid bacteria as well (Blandino et al., 2003). It has also been shown that lactic acid bacteria exhibit inhibitory activities against a wide range of pathogens by releasing organic acids like acetic, lactic, propionic and butyric acids as by-products of fermentation which lowers the pH, making the environment deleterious to the pathogens (Mensah, 1997; Holzapfel, 2002). It has also been reported that lactic acid bacteria e.g. *Lactobacillus spp* produce Proteinaceous, antimicrobial substances called bacteriocins which prevents the growth of pathogens, thereby protecting the host (Oyewole, 1997).

Our study showed that consistent intake of *Ugba* triggers an increase in the growth and population of the probiotic bacteria *Bifidobacterium spp* and *Lactobacillus spp*. This increase in growth may enhance a reduction in the population of pathogens by competitive inhibition. The rapid increases in the population of these probiotics are directly associated with beneficial effects like treatment of diarrhea, constipation and intestinal infections among others.

CONCLUSION

Probiotics play an important role in increasing host resistance to colonization by exogenous pathogenic organisms through different mechanisms. In our study, we observed that consistent intake of fermented oil bean seed was associated with increased population of probiotic bacteria and reduction in pathogenic bacteria.

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CONFLICT OF INTEREST

No conflict of interests was declared by the authors.

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