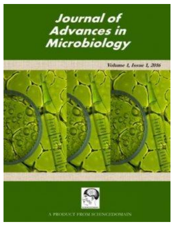

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Lactic Acid Bacteria Profile of Some Selected Food Products and Their Ability to Produce Bacteriocins

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Authors' contributions

This work was carried out in collaboration between both authors. Author GEJ designed the study, wrote the protocol, wrote the first draft of the manuscript and proof read the final draft. Author JAL carried out the analysis, performed the statistical analysis and managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Lactic acid bacteria (LAB) commonly used as starter cultures in foods is known to produce antimicrobial substances such as bacteriocins, hydrogen peroxide and organic acids. They have great potential as food biopreservatives. The present study was aimed at profiling lactic acid bacteria of some selected food products and bacteriocin production from various food products. Different food samples comprising four each of cucumber, fresh fish, fresh milk (nunu), meat, ogi, cheese, yoghurt, and tapioca making it a total of thirty two (32) was bought in area markets in Calabar Metropolis. Serial dilutions of these samples were prepared and inoculated by pour plate method on MRS medium and incubated. It was observed that the lactic acid bacteria loads ranged from 1.11±0.41 × 10^2 cfu/ml(g) for fresh milk to 4.62 × 10^3 cfu/ml(g) for cheese. As for the distinct colonies, cheese had the highest 22 while silage had the lowest 9. Others were 21 for fresh milk, 20 for fufu, 19 for ogi, 18 for yoghurt, 15 for meat, 14 for cucumber and 12 for fish. The isolates were identified based on morphological, physiological and biochemical analysis using API 50 CHL kit. One hundred and fifty five morphologically distinct colonies of lactic acid bacteria isolated from these food samples were screened for bacteriocin production. Only 26 of these were found to be bacteriocin-positive indicating their ability to produce bacteriocin. The isolates identified included

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Lactobacillus sp, *Leuconostoc* sp, *Lactococcus* spp, *Enterococcus* sp and *Pediococcus* sp. This research extends our knowledge of the ubiquitous distribution of lactic acid bacteria in foods. These bacteriocin positive isolates can, therefore, be used in food preservation and control of food borne pathogens.

Keywords: Bacteriocin; lactic acid bacteria; food products; screening; food; antimicrobial.

1. INTRODUCTION

Lactic acid bacteria are found in various food products and also in the environment. They do not have the capability to manufacture the many compounds that they need to survive and grow. Most of the nutrients must be present in the environment in which the bacteria reside. These can include food products such as carbohydrates, milk, yoghurt, fruits and vegetables, meat, fish, plant leaves and decaying organic material such as silage. Lactic acid bacteria are also of economic importance in the preservation of agricultural crops. A popular method of crop preservation utilises what is termed silage. Silage is essentially the exposure of crops (e.g., grasses, corn, alfalfa) to lactic acid bacteria. The resulting fermentation activity lowers the pH on the surface of the crop, preventing colonisation of the crop by unwanted microorganisms [1,2]. Environments in which LAB thrive are rich in sugars and protein; fats, vitamins and nucleotides are also often available. Consequently, most LAB are auxotrophic for a large number of amino acids and vitamins [3]. Fruits and vegetables were a poor source of lactic acid bacteria but large numbers were readily isolated on MRS agar from cheese, milk and meat samples [4].

Lactic acid bacteria (LAB) are well known for their production of bacteriocins. They have the potential to be used in the food and feed industry to substitute for chemical preservation [5]. Bacteriocins produced by LAB have attracted special interest as potential alternative safe commercial food preservatives. LAB have been used as food and feed preservatives for centuries, and bacteriocin-producing LAB could replace chemical preservatives for the prevention of bacterial spoilage and the outgrowth of pathogenic bacteria in food products [6].

Bacteriocins are antimicrobial peptides produced by many lactic acid bacteria (LAB), which are directed mainly to inhibit the growth of related species or species with the same nutritive requirements [7,8,9]. Bacteriocins may be produced by both gram negative and gram positive bacteria [10]. In recent years, bacteriocin producing LAB have attracted significant attention because of their GRAS status and potential use as safe additives for food preservation [11]. Some bacteriocins have been used to inhibit this pathogen in food either through bacteriocin-producing cultures [12,13] or by the addition of pure or semi pure bacteriocin preparations [14]. Many physicochemical factors seemed to affect bacteriocin production as well as its activity. Despite the fact that antimicrobial peptides have an inhibition spectrum narrower than that of antibiotics [15,16]. The bacteriocins produced by LAB have been reported to infiltrate the outer membrane of Gram-negative bacteria and to encourage the inactivation of Gram-negative bacteria in combination with other enhancing antimicrobial environmental factors, such as organic acid, low temperature and detergents materials [17,18].

The relationship between food and health has been investigated for many years, and therefore, the development of foods that promote health and well-being is a key research priority of the food industry [19]. Fruits and vegetables are an essential part of human nutrition. Unfortunately, the daily intake of fruits and vegetables is estimated to be lower than the recommendation of the World Health Organization [20], who suggest a dietary intake of 450 and 500 g of fruits and vegetables, respectively. Lactic acid fermentation of vegetables, currently used as the bio-preservation method for the manufacture of finished and half-finished foods, is an important biotechnology for maintaining and/or improving safety, nutritional, sensory and shelf-life properties of vegetables [1,21]. The production of lactic acid and other metabolites by lactic acid bacteria create unconducive environment that inhibited the growth of other organisms thereby preserving and extending the shelf-life of vegetables [1].

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of 32 food samples were bought from different locations and 4 silage samples in

Calabar. The collected samples included four samples each of cucumber, ogi, meat, fresh fish, yoghurt, fufu, silage, natural milk (nunu) and cheese. The samples were collected aseptically using sterile gloves, properly labelled and placed in food grade sampling bags. Samples were immediately placed in pre-cooled containers containing ice packs and then transported to the laboratory for analysis.

2.2 Microbial Analysis

2.2.1 Isolation of lactic acid bacteria

This was carried out using the methods of [22] and [23] with little modifications. Fifty grams of each sample was weighed and placed in sterile blender previously sterilised with 70% alcohol and rinsed with deionised water. The samples were then blended aseptically and twenty five grams of the product was placed in 225 ml of sterile peptone in sterile flask for enrichment. The homogenates were vigorously shaken for 2 to 3 minutes from which further ten-fold dilutions up to 10^{-4} were prepared. Spread and pour plate methods were used as the samples were plated on de Mann Rogosa Sharpe (MRS) agar in triplicate and incubated anaerobically.

2.2.2 Purification and maintenance of microbial isolates

The isolates were purified repeatedly via subculturing using MRS agar. Pure cultures were preserved on nutrient agar slants overlaid with paraffin oil and stored at an ambient temperature for cultural and biochemical characterisation.

2.3 Screening of Lactic Acid Bacteria for Bacteriocin Production

One hundred and fifty-five morphologically-distinct colonies were screened by the crowded plate technique against the test organisms as described by Willey et al. [24]. The test organisms used were *Staphylococcus aureus, Esherichia coli, Klebsiella pneumonia, and Streptococcus pyogenes.* Each organism was first diluted in sterile water after which 0.1 ml was spread on nutrient agar plates in triplicates and each lactic acid bacteria and fungi isolate was inoculated on the plates by agar disk diffusion method. The plates were then incubated at 28°C for 24 to 48 hours. The plates were observed at different intervals for colonies with clear zones around them. The lactic acid bacteria and fungi isolate that show zones of inhibition were regarded as bacteriocin positive isolates.

3. RESULTS

A total of 155 distinct colonies of lactic acid bacteria were isolated from the different food stuffs analysed; 20 from fufu, 18 from yoghurt, 15 from meat, 14 from cucumber, 21 from fresh fish 12 from milk, 19 from ogi, 9 from silage, and 22 from cheese. This result indicates that lactic acid bacteria are indigenous in several food stuffs. Table 1 presents the mean counts of the bacterial isolates on MRS (de Mann Rogosa Sharpe) agar. The highest lactic acid bacteria load was also recorded by fufu sample as 3.62 x 10^3 CFU/g and the least load was observed in fresh milk as 1.11 x 10^2 CFU/ml.

Tables 2 and 3 show the cultural, biochemical characterisation and sugar fermentation profile of the 26 bacteriocin positive lactic acid bacteria isolated from samples used in this study. A total of 149 distinct lactic acid bacterial species belonging to 5 different genera were identified. All the isolates were Gram positive, however, the cell morphology and arrangements as well as the pigmentation, consistency and elevation varies between the isolates. Fourteen (54%) of the isolates were creamy in colour while twelve (46%) were white. Fifteen of the isolates were cocci either in chains, clusters or tetrads while eleven were found to be rod shaped either in pairs, chains or clusters. All the isolates were catalase positive, non-motile and oxidase positive. The API profiles of these isolated LAB enabled identification of 3 strains of *Lactococcus* spp, 2 strains of *Enterococcus* spp, 14 strains of *Lactobacillus* spp, 3 strains of *Pediococcus* spp and 6 strains of *Leuconostoc* spp. API analysis results were based on the more frequently occurring forms of strains. All LAB fermenters utilised galactose, maltose glucose, fructose, mannose and lactose, which are the main fermenters.

4. DISCUSSION

The results were in accordance with previous works of several authors [25,26,27] where they all isolated lactic acid bacteria from cereals, milk, meat and vegetables. Lactic acid bacteria (LAB) are common microorganisms in foods and also constitute the natural intestinal microbiota of humans and most animals [28].

Table 1. Distribution of culturable aerobic bacteria in samples on MRS agar

S/N	Sample code	Count of culturable aerobic bacteria (cfu/mL or g)	Number of distinct colonies
1	FF	$3.62\pm0.72 \times 10^3$	5
2	YO	$1.34\pm0.59 \times 10^2$	8
3	MT	$2.80\pm0.44 \times 10^3$	6
4	CU	$4.52\pm1.25 \times 10^2$	7
5	FM	$1.11\pm0.41 \times 10^2$	10
6	FI	$1.38\pm0.69 \times 10^3$	3
7	OG	$2.41\pm0.63 \times 10^2$	6
8	SI	$3.53\pm1.03 \times 10^2$	4
9	CH	$4.62\pm0.22 \times 10^3$	8
	TOTAL		57

FF- Fufu; YO- Yoghurt; MT- Meat; CU- Cucumber; FM- Fresh Milk; FI- Fish; OG- Ogi; SI- Silage; CH- Cheese

Table 2. Primary screening of bacteria for bacteriocin production

S/N	Sample code	Number of distinct colonies	Number of bacteriocin-positive bacteria	% bacteriocin-positive bacteria per sample
1	FF	20	4	20
2	YO	18	3	16.67
3	MT	15	2	13.33
4	CU	14	3	21.43
5	FM	21	2	9.52
6	FI	12	4	33.33
7	OG	19	3	15.79
8	SI	9	2	22.22
9	CH	22	3	13.64
	TOTAL	155	26	16.77

FF- Fufu; YO- Yoghurt; MT- Meat; CU- Cucumber; FM- Fresh Milk; FI- Fish; OG- Ogi; SI- Silage; CH- Cheese

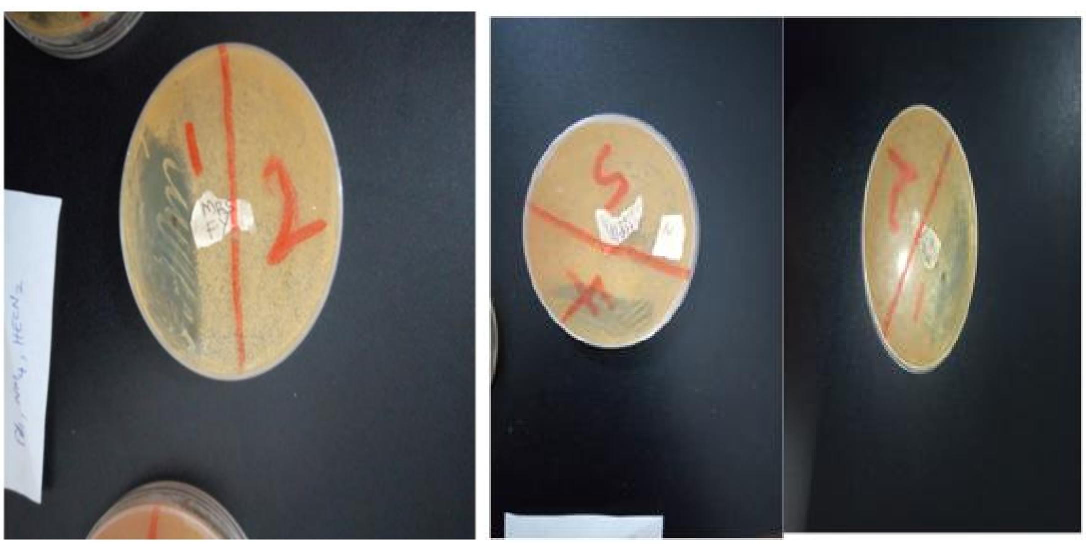

Fig. 1 and 2. Primary screening; plate showing inhibition zone as a result of crowded plate method

Table 3. Biochemical characterisation of the Bacterial isolates and API CHL 50 result

Isolate code	Catalase	Motility	Oxidase	Fructose	Tehalose	Glucose	Lactose	Sucrose	Glycogen	Mannitol	Maltose	Xylose	Melizetose	Esculin	Sorbitol	Mannose	Raffinose	Arabinose	Rhamnose	Melibiose	Ribose	Salicin	Cellobiose	Probable organism
G_1	+	-	-	-	-	+	+	-	+	+	+	-	+	-	-	-	-	+	-	+	-	-	+	*Pediococcus* spp
G_2	+	-	-	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	*Leuconostoc* spp
G_3	+	-	-	-	-	-	+	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	*Leuconostoc* spp
G_4	+	-	-	+	-	+	+	+	+	-	+	-	+	-	+	+	+	-	-	+	+	+	+	*Lactobacillus* spp
G_5	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Lactobacillus* spp
G_6	+	-	-	+	-	+	+	+	+	-	+	-	+	-	-	+	+	+	-	+	-	+	+	*Lactobacillus* spp
G_7	+	-	-	+	+	+	+	-	+	+	+	-	+	-	-	+	+	+	-	-	+	+	+	*Lactococcus* spp
G_8	+	-	-	+	+	+	+	-	+	+	+	-	+	-	-	+	+	+	-	-	+	+	+	*Lactococcus* spp
G_9	+	-	-	+	+	+	+	-	+	+	+	-	+	-	-	+	+	+	-	-	+	+	+	*Lactococcus* spp
G_{10}	+	-	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	*Lactobacillus* spp
G_{11}	+	-	-	+	-	+	+	+	+	-	+	-	+	-	+	+	+	-	-	+	+	+	+	*Lactobacillus* spp
G_{12}	+	-	-	-	-	-	+	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	*Leuconostoc* spp
G_{13}	+	-	-	+	-	+	+	+	+	-	+	-	+	-	-	+	+	+	-	+	-	+	+	*Lactobacillus* spp
G_{14}	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Lactobacillus* spp
G_{15}	+	-	-	+	-	+	+	+	+	-	+	-	+	-	+	+	+	-	-	+	+	+	+	*Lactobacillus* spp
G_{16}	+	-	-	-	-	+	+	-	+	+	+	-	+	-	-	-	-	+	-	+	-	-	+	*Pediococcus* spp
G_{17}	+	-	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	*Lactobacillus* spp
G_{18}	+	-	-	+	+	+	+	+	-	+	+	-	-	+	-	+	-	+	-	+	+	+	-	*Enterococcus* spp
G_{19}	+	-	-	-	-	+	+	-	+	+	+	-	+	-	-	-	-	+	-	+	-	-	+	*Pediococcus* spp
G_{20}	+	-	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	*Lactobacillus* spp
G_{21}	+	-	-	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	*Leuconostoc* spp
G_{22}	+	-	-	+	-	+	+	+	+	-	+	-	+	-	-	+	+	+	-	+	-	+	+	*Lactobacillus* spp
G_{23}	+	-	-	+	+	+	+	+	-	+	+	-	-	+	-	+	-	+	+	+	+	+	-	*Enterococcus* spp
G_{24}	+	-	-	-	-	-	+	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	*Leuconostoc* spp
G_{25}	+	-	-	-	-	-	+	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	*Leuconostoc* spp
G_{26}	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Lactobacillus* spp

The presence of high counts of lactic acid bacteria detected in the food samples analysed could possibly be due to the natural fermentation processes and/or the addition of lactic starters during the processing of such products [29,30]. Fluctuated numbers of LAB detected in different types of the investigated food samples may be depended on the methods used in handling and storage of this product as well as the hygienic status of environments where they are retailed [29]. LAB particularly those belonging to beneficial and non- pathogenic bacteria, play essential role in dairy industry due to the tremendous level of human consumption of several important fermented products, mainly cheese and acidified or fermented milks [31].

The isolation of such LAB species from foods in this study is in line with the work of many researchers in different countries. In Morocco [32] isolated a bacteriocin-producing *L. lactis* sub sp. *Lactis* Strain from a soft white cheese effective against *L. monocytogenes.* In Senegal, [11] isolated 220 LAB strains from traditional fermented foods. Twelve of them were bacteriocin producing isolates of which two isolates were identified as *L. lactis* subsp *lactis* and *Enterococcus faecium*. In Nigeria, [33] identified *L. plantarum* and *Lactobacillus brevis* as the most dominant bacteriocin-producing LAB strains isolated from West African soft cheese however *Lactococcus lactis*, *Streptococcus lactis* and *Lactobacillus fermentum* were less dominant. Also, [34] isolated different lactic acid bacteria from fermenting garden egg. In France, [35] identified six bacteriocin-producing LAB isolates from cheese.

From the 155 colonies screened, only 26 isolates (16.77%) were found to be bacteriocin positive indicating their ability to produce bacteriocin as shown on Table 2. This shows that not all LAB are capable of producing bacteriocin. The actual prevalence of bacteriocin positive Strains in the food samples analysed as shown in Table 2 are much higher than those reported by other findings. Sharpe [36] reported detecting 8.7% bacteriocinogenic strains among 92 isolated organisms from fresh-cut vegetable products, whereas [37] screened 12,700 LAB isolates from milk and meat products and found only 35 exhibited bacteriocin production. Also, [38] screened 3000 colonies of LAB isolated from traditional fermented foods and only one colony was found to produce inhibitory zone. The high incidence of bacteriocin producing LAB in these foods suggests that they may represent an abundant source of potentially useful bacteria. A similar observation was made by [39] in raw and malted cereals. LAB is also abundant contaminants in many other foods such as milk, meat and vegetables [40,26]. The high incidence of LAB, including bacteriocinogenic strains in cereals has significant implications for the quality, safety and shelf life of these and related foods. Therefore, the choice of food source is important for the successful isolation of bacteriocinogenic organisms.

5. CONCLUSIONS

This work extends our knowledge of the ubiquitous distribution of lactic acid bacteria in foods and provides a further argument that consumers may be ingesting lactic acid bacteria or their byproducts on foods they already eat. Despite the heavy emphasis on food safety in the past decade whereby antimicrobials are added/sprayed onto raw or processed meats, vegetables and produce for the interest of “pathogen reduction”, there does not seem to be a scarcity of lactic acid bacteria on foods, providing researchers a broad array of antimicrobials for potential use as food preservatives.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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