

Anti-Bacterial Activities of Crude Cell Free Supernants of Lactic Acid Bacteria from Wara (Nigerian Soft Cheese).

Ibrahim TA^{1*}, and Falegan CR²

¹Department of Food Science and Technology, Rufus Giwa Polytechnic, PMB 1019, Owo, Ondo State, Nigeria.

²Department of Microbiology, Ekiti State University, Ado Ekiti, Nigeria.

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*For Correspondence

Department of Food Science and Technology, Rufus Giwa Polytechnic, P.M.B 1019, Owo, Ondo State, Nigeria.

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ABSTRACT

Crude cell free supernant (CFSs) of lactic acid bacteria from wara were isolated and tested for their invitro antibacterial activities against selected clinical pathogenic bacteria such as *Salmonella sp*, *Staphylococcus sp*, *Bacillus sp*, *E.coli* and *Klebsiella sp*. Wara samples were collected from hawkers in sterile container and grown on the surface of nutrient agar and MRS agar at 37°C for 24 hours. The CFSs were obtained by centrifugation. The antibacterial activity of the CFS was carried out using agar well diffusion assay. The zones of inhibitions were 0.6±0.1, 1.5±0.03, 0.0±0.0, 0.0±0.0 and 0.8±0.0 respectively. *Staphylococcus aureus* was the most susceptible with the diameter of inhibition zone range between 1.5±0.03mm followed by *Klebsiella sp* 0.8±0.0mm/ml, *Salmonella sp* 0.6±0.1mm. CFSs showed no activity against *E.coli* and *Bacillus sp*. These data suggest that the CFSs could be used as a good source of nutraceutical.

INTRODUCTION

The term probiotic was introduced defined by [22] to describe growth promoting factors produced by microorganisms. Probiotics are defined as live microorganisms in foodstuffs which when consumed at certain levels in nutrition, stabilizes the gastrointestinal tract microflora thereby conferring health benefits on the consumer. Probiotics are selected from the strains most beneficial for the host intestinal bacteria. Members of the genera *Lactobacilli*, *Bifidobacterium* and the yeast *Saccharomyces boulardi* are mainly used. Other organisms are also applied such as members of the genera *Lactococcus* and *Enterococcus* [9]. The main invitro selection criteria for potential probiotic strains are acid and bile resistance activities, indicating the ability of the organisms to survive the passage through the gastrointestinal tract [1]. Production of antimicrobial compounds such as bacteriocins, lactic acid, acetic acid and competition for nutrient and adhesion sites may contribute to the control of intestinal pathogens [16]. The relationship between dairy foods and health effects has been investigated for many years [2]. In recent years, numerous studies have been undertaken to obtain scientific evidence for beneficial effects of fermented dairy products containing specific probiotic strains [4].

In Nigeria, approximately 90% of the dairy cattle belong to the fulani pastoralist who process surplus fresh milks to nutritious milk products such as nono, kindirmo, manshanu and wara. Wara has been classified as a natural cheese made from milk. The soft wara cheese product in Nigeria farms especially in the northern part makes use of local ingredients. The vegetable renet used for the production is made from a native plant *Calotrophis procera* which can be cultivated all round the year [14]. Wara has an average shelf life of 2-3 days when stored in whey at ambient temperature (approximately 28°C) [14] although its storage at lower temperature had a little effect on the microbial counts [14]. The wara is usually deep fried in vegetable oil near the end of its shelf life in order to extend the shelf life. Due to lack of household refrigeration facilities in Nigeria and West Africa, attempts have been made in the recent past to include starter culture or various chemical preservatives such as propionic acid, sodium benzoate and ascorbic acid in the production of wara [19]. Some of these preservatives have been shown to be effective in inhibiting mesophilic and psychotrophic bacteria as well as coliforms. However, the preservatives are not easily accessible to the local cheese processors in West Africa. This work was carried out to present a new data about the therapeutic role of crude cell free supernants (CFSs) of the isolated Lactic acid bacteria from wara on selected clinical bacterial isolates.

MATERIALS AND METHODS

Collection of Wara Samples

Different wara samples (100) were aseptically collected from two different selling points in Owo, Ondo State, Nigeria in sterilized containers and transported on ice pack from the collection points to the laboratory for analysis.

Selective Isolation and Identification of Lactic Acid Bacteria

Eleven grams of wara sample were transferred aseptically into sterile bottle containing 99ml of sterile peptone water and mixed thoroughly. Serial dilutions ($10^3/10^5$) were made for each and 1ml of the appropriate dilution was transferred into MRS agar and incubated at 35°C for 48 hours. The identification was carried out using conventional methods of such as colony morphology, cultural characteristics, biochemical tests, sugar utilization as described by [18]. API 50 CH strip was used as confirmatory method for the selected isolates.

Test Bacteria Isolates

The test bacteria isolate from surface water samples used for drinking in Owo town. They were *Staphylococcus sp*, *Salmonella sp*, *E-coli*, *Klebsiella sp*, *Bacillus sp*, and *Serratia sp*. They were maintained in MacCarthy bottle at 4°C prior to assay.

Preparation of Crude Cell-Free Supernants

Crude CFSSs were prepared by growing the identified isolate in MRS broth at 37°C for 24 hours. The CFSSs were obtained by centrifugation at 6000rpm for ten minutes at 4°C. This was done according to modified method of [13]. The CFSSs were stored at 4°C prior to use.

Antibacterial Assay of the CFSSs.

This was carried out by agar well diffusing assay as described by [25]. The test bacteria isolates were plated in petri dishes on Muller-Hinton agar and wells of about 6mm diameter were poured on the wells. The plates were incubated at 37°C for 24 hours. The zones of inhibition were measured in mm. this was done in triplicate and the mean and the standard deviation of the results were recorded.

RESULT AND DISCUSSION

Table 1: Characteristics of the probiotics from Wara samples

Cell from	Tests		
	spherical	spherical	Rods
Cell arrangement	pairs/chain	pairs	chain
Grams reaction	+ve	+ve	+ve
Glucose utilization	+ve	+ve	+ve
Litmus test in milk	no reduction	no clotting	variable
Growth at pH of 3.5-4.5	-ve	+ve	+ve
Growth at 35-45°C	+ve	+ve	+ve
Proposed LAB	<i>Streptocococi</i>	<i>Enterococci</i>	<i>Lactococci</i>

Table 2: Antibacterial activity of CFSS against selected pathogen isolated from water.

Bacterial isolates	CFSSs concentration(v/v) / Diameter of inhibition zones(mm)				
	25	50	100	150	200
<i>E. coli</i>	Nil	0.4±0.01	0.6±0.08	1.3±0.06	1.8±0.02
<i>Serratia mercerscens</i>	0.8±0.02	0.9±0.03	1.4±0.06	1.8±0.04	2.6±0.03
<i>Pseudomonas aeruginosa</i>	Nil	0.3±0.01	0.8±0.02	1.3±0.01	2.1±0.04
<i>Bacillus cereus</i>	0.6±0.01	1.2±0.06	1.6±0.01	2.6±0.08	3.2±0.01
<i>Staphylococcus aureus</i>	1.2±0.03	1.5±0.02	1.8±0.01	3.2±0.03	4.6±0.06
<i>Salmonella typhi</i>	Nil	0.4±0.02	0.6±0.12	1.4±0.01	2.4±0.03

Table 2 Showed the antibacterial activity of the CFSSs isolated from wara on selected pathogenic bacteria. The data in the table represent the mean of triplicate result and standard deviation of such results. It showed that *Staphylococcus aureus* was the most susceptible of the tested organisms with inhibition zone range of 1.2±0.03 to

4.6±0.06 (mm), followed by *Bacillus cereus* and *Serratia mercerscens* with inhibition zone range of 0.6±0.03 to 3.2±0.01 (mm) and 0.8±0.02 to 2.6±0.03 (mm) respectively. *E.coli* and *Pseudomonas aeruginosa* were less susceptible with inhibition zones of 0.4±0.01 to 0.8±0.02 (mm) and 0.3±0.01 to 2.1±0.04 (mm) respectively.

In Nigeria, there are a lot of traditional dairy products in which 'wara' is one of them, are usually produced from unpasteurized cow milk which has been great potential food sources for isolating strains of probiotics. These foods can be regarded as functional after comprehensive evaluation. It has been discovered that these foods are complex ecosystem dominated mainly by lactic acid and bacteria [1]. The relativity between dairy foods and health effects has been investigated for many years. During recent years tremendous studies has undertaken to obtain scientific evidence for beneficial effects of fermented dairy products containing specific probiotics strain [22]. In this study, various species of lactobacillus were isolate. A total of 474 isolate belonging to three different genera were identified as shown in table 1. These were *Lactobacillus sp*, *Enterococcus sp* and *Streptococcus sp*, The result of LAB count shows that fermentation does occur (even though minimal) in wara production and mainly carried out by lactic acid bacteria in uncontrolled condition of fermentation. Similar results were obtained by [17,6,5] just to mention few.

In Africa, the concept of probiotics and the production of probiotic foods are equally gaining acceptability especially with the knowledge that some traditional foods containing beneficial bacteria (Lactic acid bacteria) have been an acceptable diet among the local people. Antimicrobial activity is one of the more important selection criteria for probiotics. Antimicrobial activity targets the enteric and undesirables pathogen. The antimicrobial effects of lactic acid bacteria are formed by producing some substance such as organic acid, CO₂ hydrogen peroxide, diacetyl, low molecular weight antibacterial substance and bacteriocin [21]. Studies on Yakult and Ski D milk products showed that they produce probiotic that inhibited the growth of pathogens such as *Staphylococcus sp*, *Pseudomonas sp*, *Serratia sp*, *Salmonella typhi*, *Bacillus sp*, *E-coli*, *Klebsiella sp* and *Candida sp*. Neslac Probiotic was inhibitory to *E-coli*, *Staphylococcus aureus* 20. Also, LAB isolated from Burkinafaso fermented milk showed antimicrobial activity against *E.coli*, *Staphylococcus sp*, *Klebsiella sp*, *Bacillus sp*, and *Salmonella sp*. [12] Davidson et al [7] also investigate the lactobacillus sp isolated from Vaginal swabs for bacteriocin production. It was found out that the isolated lactobacilli had antimicrobial activity against *Staphylococcus aureus*, *proteus vulgaris*, *E-coli*, and *Candida albicans*.

The antibacterial activity of the CFSs as shown in table 2 showed the antibacterial of the CFCs was concentration dependent. Five different concentrations 25mg/ml, 50mg/ml, 100mg/ml, 150mg/ml and 200mg/ml. *E.coli* was the most resistant while *Staphylococcus aureus* the was the most susceptible followed by *Bacillus* and *Serratia sp*. The documentation of probiotic bacteria from Wara is scanty. There is no publication carried out to study the therapeutic, functional role or even the bacteriocin activity of probiotics from Wara. The mechanism by which probiotics beneficially affect human health are divided into many categories, including strengthening of the intestinal barriers modulation of the immune response, and antagonism of pathogens either by the production of antimicrobial compound or through competition for mucosal binding sites [10]. Although there is suggestive evidence for each of these functional claims, the molecular details these mechanisms remain entirely unknown [8]. The presence of inhibition zones of the CFSS assumed a good therapeutic indication of the species of the lactobacillus acting as probiotic. Similar results were reported in other studies on several species of lactobacillus [15]. Based on these results, it is most likely that antagonistic activity was caused by production of organic acid and reduction of pH, although inactivation of bacteriocin during neutralization cannot be ruled out. It is also possible that antimicrobial substance is membrane associated as noted by Ebrahim et al [14]. Jacobson [3] reported that a antimicrobial activity by lactic acid bacteria from African fermented foods in many previous studies were due to the production of lactic acid and low pH. Hydrogen peroxide (H₂O₂) has also been targeted to enact antimicrobial effect [8]. The virulence potential of pathogen is reduced by inhibition activity at the CFSs as shown by the diameter of inhibition zone. The results of this study are quite significant because the pathogens inhibited by the CFSs were clinical isolates from drinking water sample of great medical importance. The contemporary means of management of the disease carried by these pathogens include antibiotic therapy which is a selector for resistance strains. The present study therefore shows a potential application of indogenous strains of lactobacilli from Wara in ameliorating a major global health problem and reducing antibiotic usage.

CONCLUSION

This present work has shown that several *Lactobacillus* species were associated with wara and the result of the antibacterial activity of their crude cell free supernatant showed the possible probiotic potential of the African soft cheese.

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