Global Journal of Medical research: C
Microbiology and Pathology
Volume 14 Issue 4 Version 1.0 Year 2014
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Effect of Varied Culture Conditions on Bacteriocin Production of Four Lactobacillus Species Isolated From Locally Fermented Maize (Ogi)

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Materials and methods: Four (4) isolates of bacteriocin producing lactobacillus species (L. lactis, L. fermentum, L. casei and L. plantarum) with antibacterial activity against Salmonella typhimurium (ATCC 14028) and Shigella dysenteriae (ATCC 23351) were subjected to varied growth medium conditions. Bacteriocin production was tested at different physical and cultural conditions such as temperature (25, 30, 35 and 400C), pH (5, 6, 7 and 8), sodium chloride (NaCl) concentration (2, 4, 6 and 8%) and incubation duration (12, 24, 48 and 72 hours).

Aim: To isolate and identify LAB species from fermented maize (Ogi) and to determine the effect of varied culture conditions on bacteriocin production and antibacterial activity against indicator organisms.

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GJMR-C Classification : *NLMC Code: QW 50*

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Results: The optimum bacteriocin production judged by their different zones of inhibition was recorded at temperature, 30^{0}C and then 35^{0}C. There were significant differences between all the incubation temperatures at $P<0.05$. Duration of incubation showed highest bacteriocin activity after 72 hours. Furthermore, optimal conditions for bacteriocin production were observed to be highest at pH 6.0 followed by 5.0 and then in 2% NaCl concentration. There were significant differences between the zones of inhibition of bacteriocins produced against the indicator organisms at various media pH and salt concentrations at $P<0.05$.

Conclusion: These bacteriocins may have a potential use as food preservative and may help in improving the gastro-intestinal tract by fighting off pathogenic bacteria.

Keywords: bacteriocin, optimum, varied, culture, condition, fermented, maize, ogi.

I. Introduction

Food is any substance or mixture of substances both solid and liquid, which are intended for human consumption or ingestion for their nutritional support for the body or pleasurable benefits. It usually consists of plant or animal origin, which contains essential nutrients such as carbohydrates, fats, proteins, vitamins or minerals and is ingested and assimilated by an organism to produce energy, stimulate growth and maintain life [1] [2].

The lactic acid bacteria (LAB) are a group of Gram positive bacteria, non–respiring, non–spore forming, cocci or rods, which produce lactic acid as the major end product of the fermentation of carbohydrates. They are the most important bacteria in desirable food fermentations, being responsible for the fermentation of sour dough bread, sorghum beer, all fermented milk, cassava (to produce garri and fufu) and most "pickled" (fermented) vegetables [3] [4]. Lactic acid bacteria occur naturally in several raw materials like milk, meat and flour used to produce foods. LAB is used as natural or selected starter cultures in food fermentations in which they perform acidification due to production of lactic acids. Protection of food from spoilage and pathogenic microorganisms by LAB is through producing organic acids [5]. The LAB produces an array of antimicrobial substances (such as organic acids, diacetyl, acetone, hydrogen peroxide, reuterin, anti-fungal peptides and bacteriocins [6] [7] [8]. Bacteriocins are ribosomally synthesized antimicrobial peptides or proteins [9].

Ogi (Akamu) is a product of fermented maize (Zea mays) widely eaten in Africa [10] [11]. Similar maize preparations in Ghana are referred to as "Akana" or "Kenkey". Ogi is often marketed as a wet cake formerly wrapped in leaves but presently in transparent polythene bags. Gelatinized Ogi (a porridge) called "pap" is mainly used as a breakfast meal for adults and weaning food by low income earners who cannot afford the more expensive imported weaning foods [12]. In most parts of Africa especially in Nigeria, children are fed with mashed adult foods. These foods are bulky and this therefore reduces food intake by a child, often resulting in malnutrition. The development of nutritionally balanced calorie less dense, low bulk and easily digestible weaning food becomes necessary. This involves the use of simple but time consuming traditional technology called fermentation [13]. The

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traditional fermentation method employed in Ogi production is a wild process and microorganisms are not controlled [14]. Microbiological analyses have shown the presence of several genera of bacteria, moulds and yeasts in the fermented maize product-Ogi [15] [16].

In the present study, different culture conditions were adjusted for bacteriocin production using *Lactobacillus* isolates from locally fermented maize (Ogi) to determine optimal fermentation conditions for bacteriocin production.

II. Material and Methods

a) Fermented Products

Ten (10) fermented maize (Ogi) samples bought from Oshodi and Odo markets, Lagos State, Nigeria were analyzed.

b) Test Organisms

Pure strains of pathogenic gram negative bacteria responsible for food infections; *Salmonella typhimurium* (ATCC 14028) and *Shigella dysenteriae* (ATCC 23351) were obtained from the Nigerian Institute of Medical Research laboratory (NIMR) Yaba, Lagos, Nigeria and maintained on agar slants at 4^0C in the refrigerator.

c) Isolation of lactic acid bacteria from Ogi

One (1) gram of a 72hrs fermented Ogi was transferred into 5ml peptone water (Merck, Germany) and serially diluted (10 fold dilutions). Then 1ml of each of the dilution was aseptically transferred into sterile Petri dishes (Pyrex and Anumbra) and 15ml of de Man Rogosa Sharpe (MRS) medium (Merck, Germany) was added using pour plate technique then incubated at 370C for 48hrs in an anaerobic flask (Oxoid). After incubation, colonies with different morphologies were randomly selected using a flamed platinum wire loop, streak plated and sub – cultured on MRS agar plates to obtain pure colonies. All isolates were examined for Gram reaction, production of catalase and oxidase activity.

d) Identification of LAB Isolates

Isolates were identified using the following tests: ammonia production from arginine, CO2 production from glucose and growth at different pH values, growth at different NaCl concentrations and carbohydrate fermentation. LAB isolates were tested for characteristics of Gram staining, cell morphology, colony morphology, motility, carbon dioxide production from glucose, growth at 100C and 450C, growth at pH of 4.4 and 9.6, growth in 6.5% and 18% NaCl, catalase reaction by 3% hydrogen peroxide and carbohydrate fermentation [17].

e) Detection of inhibitory activity of crude bacteriocin from selected isolates

Selected LAB isolates were grown in MRS broth at 37°C for 24 hrs. Cell free supernatant of each isolate was obtained by centrifugation at 3,000xg at 4°C for 20 min. The supernatant was adjusted to pH 6.5 with 1M NaOH and subsequently filter sterilized through a 0.2μm membrane filter (Whatman, Germany). Inhibitory activity was determined using agar well diffusion assay [18]. Inhibitory effect of the hydrogen peroxide in the supernatant was eliminated by reacting with 5mg/ml catalase added. Suitable agar medium containing 1% agar (45°C) was inoculated with each of the two indicator strains. Agar wells of 5 mm diameter were cut and the filter-sterilized supernatant (20μl) was added into each well. The plates were incubated at 37°C for 24hrs. The inhibition zones around the wells were measured.

f) Optimization of Culture Conditions

The selected lactic acid strains were subjected to different culture conditions to derive optimum conditions for bacteriocin production

g) Effect of varying culture conditions on bacteriocin activity

To study the effect of varying culture conditions, growth and bacteriocin production was estimated at varied temperatures (25, 30, 35, and 400C) pH (5.0, 6.0, 7.0 and 8.0), sodium chloride (NaCl) concentrations (2.0, 4.0, 6.0 and 8.0% w/v) and duration of incubation (12, 24, 48, and 72hrs) in MRS broth. All samples were collected after 48hrs, except for those measuring incubation time effects before inhibitory activity was determined by agar well diffusion assay as described above.

h) Data presentation and statistical analysis

Data were represented as means ± standard error of mean as well as bar charts. Two – way analysis of variance and Bonferroni's multiple comparison tests using GraphPad Prism (version 6.0) software were used to analyze data. Values were considered significant when $P<0.05$.

III. Results

a) Isolation of lactic acid bacteria

In this study, Lactobacillus strains producing antimicrobial compounds were isolated from fermented maize (Ogi). Out of a total of seven (7) isolates that met basic characteristics of Lactobacilli, only four (4) showed antibacterial activity against both indicator organisms. They include *L. lactis, L. fermentum, L. casei and L. plantarum*.

b) Optimization of Culture Conditions

The broth medium containing each isolate was incubated at various temperatures; 25.0, 30.0, 35.0 and

40.0^{0}C and the bacteriocin harvested were tested against *Salmonella typhimurium* and *Shigella dysenteriae* (Figure 1 and 2 respectively).

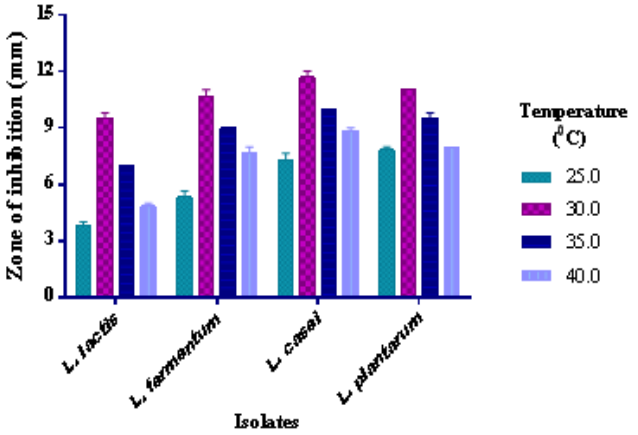

Fig. 1 : Zone of inhibition (mm) of crude bacteriocin from different *Lactobacillus* isolates cultured at varied temperatures (^{0}C) against *Salmonella typhimurium*

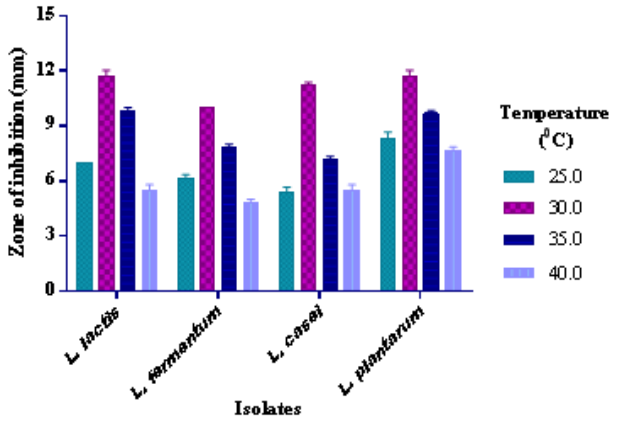

Fig. 2 : Zone of inhibition (mm) of crude bacteriocin from different *Lactobacillus* isolates cultured at varied temperatures (^{0}C) against *Shigella dysenteriae*

The effect of varied incubating duration (Hrs) on bacteriocin production was noted as different zones of inhibition were observed among the various isolates against the indicator organisms (Figures 3 and 4).

The effect of varied medium pH and Sodium chloride concentration on bacteriocin production was affected as different zones of inhibition were observed among the various isolates against the indicator organisms (Figures 5 and 6; Figures 7 and 8) respectively

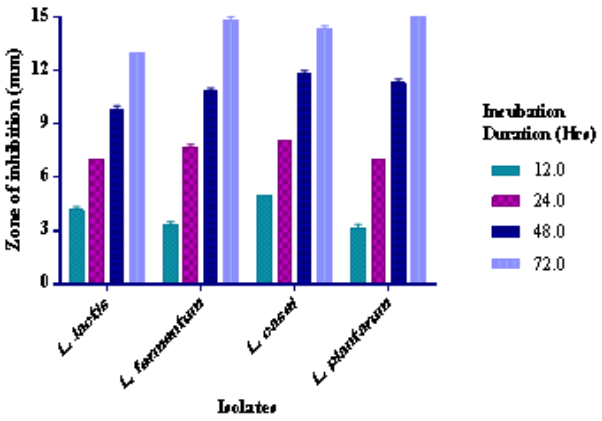

Fig. 3 : Zone of inhibition (mm) of crude bacteriocin from different *Lactobacillus* isolates cultured at varied incubation durations (Hrs) against *Salmonella typhimurium*

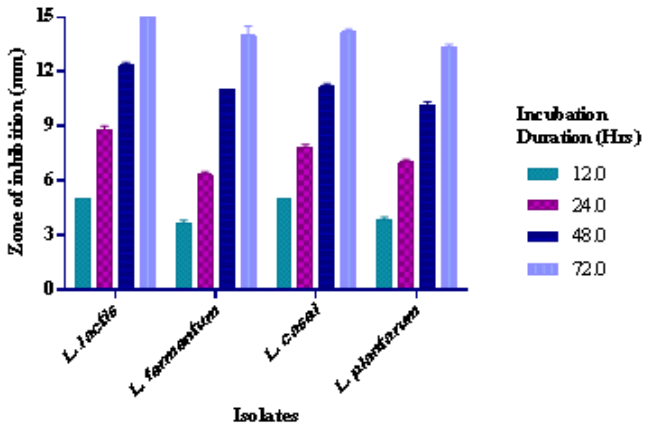

Fig. 4 : Zone of inhibition (mm) of crude bacteriocin from different *Lactobacillus* isolates cultured at varied incubation durations (Hrs) against *Shigella dysenteriae*

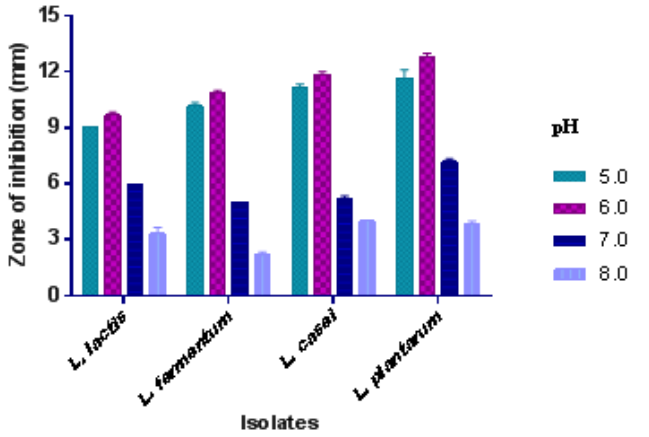

Fig. 5 : Zone of inhibition (mm) of crude bacteriocin from different *Lactobacillus* isolates cultured at varied media pH against *Salmonella typhimurium*

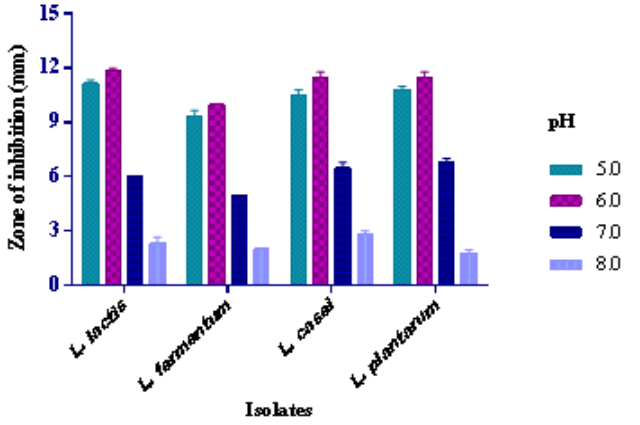

Fig. 6 : Zone of inhibition (mm) of crude bacteriocin from different *Lactobacillus* isolates cultured at varied media pH against *Shigella dysenteriae*

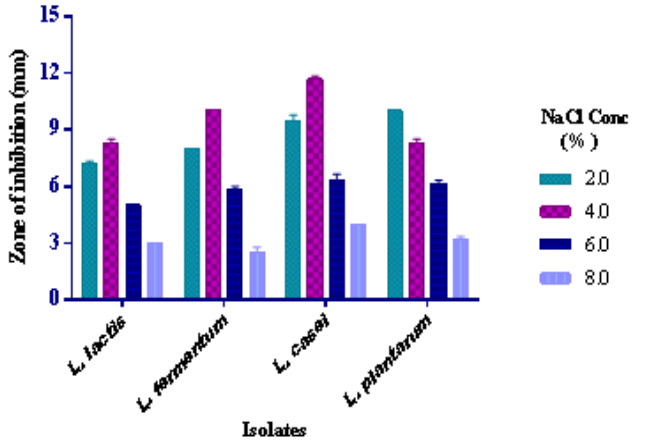

Fig. 7 : Zone of inhibition (mm) of crude bacteriocin from different *Lactobacillus* isolates cultured at varied media sodium chloride concentration against *Salmonella typhimurium*

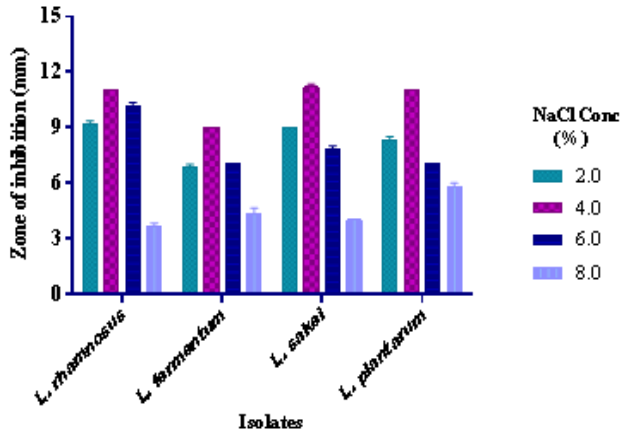

Fig. 8 : Zone of inhibition (mm) of crude bacteriocin from different *Lactobacillus* isolates cultured at varied media sodium chloride concentration against *Shigella dysenteriae*

IV. Discussion

These identified *Lactobacillus* species were in agreement with those earlier identified from similar fermented food products by [19]. The isolates were then tested for antibacterial activity against the indicator organisms (*Salmonella typhimurium* and *Shigella dysenteriae*). The bacteriocin activity of the isolates that showed antibacterial activities were further tested after the culture conditions were varied to determine the effect of different cultural conditions on the antibacterial action of bacteriocins produced. Parameters such as incubation duration and temperature, media pH and salt concentrations were varied.

The effect of varied incubating temperatures on bacteriocin production by the isolates in de Man Rogosa Sharpe broth was determined by its antibacterial activity against the indicator strains. The result showed that antibacterial activity was highest at 30.0^{0}C followed by 35.0^{0}C as seen in figures 1 and 2. There were significant differences between the different zones of inhibition produced for all the incubating temperatures (at $P<0.05$). This was in agreement with the work of [20] and [21], which showed that bacteriocin production was affected by different incubating temperatures. The maximum bacteriocin activity recorded at 30^{0}C suggests that ambient growth temperature is most ideal for bacteriocin production by *Lactobacillus* species.

The effect of varied incubating duration (Hrs) on bacteriocin production was affected as different zones of inhibition were observed among the various isolates against the indicator organisms (Tables 3 and 4). Optimum bacteriocin production was observed after 72 hours judged by the zones of inhibition against the indicators. There were observable reduction bacteriocin activities as incubation time dropped. There were significant differences between the various incubation times (at $P<0.05$). This result was in complete agreement with [22], which showed that incubation time affects bacteriocin production.

The results obtained in this study regarding bacteriocin activity from media incubated at varied pH values (Figures 5 and 6) showed optimum activity at pH 6 followed closely by pH 5 for both indicator organisms. There were clear significant differences between pH 7 and 8 but not in pH 5 and 6 as both showed similar zones of inhibition for all the isolates against indicator organisms. This result was consistent with the reports of [23], which showed influence of pH on growth of vaginal Lactobacilli. This pH tolerance is an extremely important feature since the isolates have the ability to survive, grow and produce bacteriocins under acidic and alkaline conditions.

Every microorganism has a minimal, a maximal and an optimal pH for growth and metabolism. Microbial cells are significantly affected by the pH of their

immediate environment because they apparently have no mechanism for adjusting their internal pH.

Furthermore, the effect of varied medium percentage sodium chloride (NaCl) concentration on bacteriocin production and activity was also evaluated. Highest zones of inhibition and consequent optimum bacteriocin production was observed at NaCl concentration of 4%, but started reducing as salt concentration increased further (Figures 7 and 8). There was significant difference between zones of inhibition obtained at all the salt concentrations. This was in agreement with [22] who studied cultural parameter for bacteriocin production.

Besides the strong acid medium in the stomach, the probiotic microorganisms taken orally have to defend against the bile salt in the gastrointestinal tract [24]. Hence, bile tolerance is considered to be one of the important properties required for high survival and as a consequence of probiotic activity. The decrease in bacteriocin production as salt concentration increased could be attributed to stress on the isolates.

Microbial food safety is an increasing public health concern worldwide [25] [26] and many gram negative bacteria like *Escherichia coli, Salmonella* serovars, *Campylobacter* species, *Shigella* species etc, have been implicated in food borne diseases [19]. Alternate methods for controlling pathogenic bacteria by the production of antimicrobial peptides called bacteriocins are now highly considered. Bacteriocins from lactic acid bacteria have attracted much attention and have been the subject of intensive investigation due to their ability to act as a bio-preservative agent, which led to their incorporation into foods, particularly in the dairy foods and also in human therapeutics [27] [28] [29] [30].

V. Conclusion

This study thus suggests the selection of bacteriocin – producing *Lactobacillus* strains as starter cultures for controlled fermentations at optimum cultural conditions. Bacteriocins from *lactobacillus* species harnessed under different culture conditions have shown different antimicrobial potencies. These findings could be applied in the food and pharmaceutical industries to further enhance maximum bacteriocin production at optimal levels to replace conventional antibiotics in combating pathogens that are vastly acquiring antimicrobial resistance.

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