British Microbiology Research Journal
9(3): 1-9, 2015, Article no.BMRJ.13471
ISSN: 2231-0886

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Effects of PH and Storage Temperatures on Antibacterial Activity of Bacteriocin Produced by Lactic Acid Bacteria Isolated from OGI

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Authors' contributions

This work was carried out in collaboration between all the authors. Author REO designed the study and prepared the initial manuscript. Authors GE and SAE managed the analysis and did the literature searches, while authors JOI and SAE did the preparation of the final manuscript. All authors read and approve the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2015/13471
Editor(s):
(1) Raúl Rodríguez-Herrera, Autonomous University of Coahuila, México.
Reviewers:
(1) Anonymous, Monash University, Malaysia.
(2) Anonymous, Chlef University, Algérie.
(3) Anonymous, Lahore College for Women University, Pakistan.
(4) Anonymous, Sakarya University, Turkey.
Complete Peer review History: http://sciencedomain.org/review-history/9945

Short Communication

Received 18th August 2015
Accepted 11th May 2015
Published 29th June 2015

ABSTRACT

Aims: Lactic acid bacteria are important organisms recognized for their fermentative ability. They produce various compounds including bacteriocins which are proteinaceous antimicrobial compounds. The purpose of this study was to evaluate the lactic acid bacterial content of ogi, extract crude bacteriocin from the lactic acid bacterial isolate(s) and determine the effects of pH and storage temperature on the antimicrobial properties of the crude bacteriocin extract.
Study Design: The study was designed to isolate and characterise lactic acid bacteria from ogi, thereafter extract crude bacteriocins from the isolates and then determine the antibacterial activities of the bacteriocins so extracted against some known indicator organisms.
Place and Duration of Study: Ogi samples were purchased from vendors who hawk it around the Benson Idahosa University campus and the study was done at the Department of Basic Sciences, Faculty of Basic and Applied Sciences, Benson Idahosa University, Benin City, Nigeria; between

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October 2012 and June 2013.
Methodology: Preliminary isolation and characterization of lactic acid bacteria was done using standard microbiological methods after which the bacteriocins were extracted by propagating in 500ml MRS broth (pH 7.0 glucose 0.25% w/v) and incubated for 72 h at 30ºC under anaerobic conditions. Extract was obtain by centrifuging cultures at 10,000 rpm for 20 min. Antimicrobial activity of the extract was determined using agar well diffusion method. Indicator organisms utilized were; *Escherichia coli* and *Staphylococcus aureus*. The effects of pH and storage temperatures of the crude bacteriocin, on the antimicrobial properties were determined using standard methods.
Results: *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus brevis*, *Lactobacillus fermentum* and *Lactobacillus jensenii* were the lactic acid bacteria species isolated and identified from the Ogi samples. *Lactobacillus plantarum* had the highest zone of inhibition during the screening test for antibacterial activity and was used to produce the bacteriocins used for the subsequent tests. At pH 2, there was a high antimicrobial activity but at pH 10, there was no antimicrobial activity. Crude bacteriocin extract stored at -20ºC also showed the highest antimicrobial activity.
Conclusion: This study has established that Ogi is a viable source of several lactobacilli which are capable of producing several antimicrobial compounds such as bacteriocins. It also established that bacteriocins recovered from *Lactobacillus plantarum* have a potent antimicrobial activity against a variety of both diarrhoeagenic and spoilage bacterial.

Keywords: Ogi, lactic acid bacteria; bacteriocin; pH; storage temperature; antibacterial.

1. INTRODUCTION

Lactic acid bacterial are important organisms recognized for their fermentative ability as well as their health and nutritional benefits [1]. They produce various compounds such as bacteriocin or bacteriocidal proteins during lactic acid fermentation [2]. Lactic acid bacteria are Gram positive bacteria, with low guanine and cytosine content, acid tolerant, non-sporulating, non-respiring rod or cocci that are associated by common metabolic and physiological characteristics. Bacteriocins are produced by several Lactic acid bacteria strains and this is to the disadvantage of other spoilage and pathogenic microorganisms. Bacteriocins are proteinaceous antibacterial compounds and exhibit bacteriocidal activity against species closely related to the producer strain [3]. Many bacteriocins are active against food-borne pathogens especially against *Listeria monocytogenes*. *Leuconostoc mesenteroides* L124 and *L. curvatus* L442 isolated from dry fermented sausages, produce bacteriocin antagonistic towards closely related species and pathogens [4]. An isolate of *L. mesenterioides* sub sp. cremoris was also found to produce a bacteriocin- like inhibitory compound against the lactic acid bacteria of wines [5]. Other bacteriocins of Lactobacilli have been reported to be effective against closely related species of mesophilic *Lactobacillus* and therefore considered as potential natural food preservatives [3]. Several types of bacteriocins from food-associated lactic acid bacteria have been identified and characterized, of which the important ones are nisin, diplococcin, acidophilin, bulgarican, helveticins, lactacins, and plantaricins [6]. Three of the most important aspects in the study of bacteriocins are their production, characterization and purification. Most bacteriocins produced by Gram-positive bacteria are from lactic acid bacteria [7]. Bacteriocins produced by lactic acid bacteria are divided into four well-defined groups [8]; class I, corresponds to modified bacteriocins, known as lantibiotics; class II, bring together small, heat-stable, cationic, amphiphilic and hydrophobic peptides without modified amino acid residues; class III, are larger heat-labile bacteriocins, and class iv are the cyclic bacteriocins.

Ogi is popular in Nigeria and in most of West Africa. It is an acid fermented cereal gruel or porridge made from maize (*Zea mays*) or corn; sorghum (*Sorghum vulgaris*) or millet (*Pennisetum typhoideum*). The choice of grain depends on preference and ethnicity. The microorganisms in ogi have been isolated and identified [9]. The predominant lactic acid bacterium in the ogi fermentation is *L. plantarum* and is also responsible for the production of lactic acid, the main acid in ogi. In ogi, the acidity is usually below pH 4. Most pathogenic microorganisms found in food cannot survive this low pH, hence, Lactic acid fermentation of food has been found to reduce the risk of having pathogenic microorganisms grow in the food [10].

Ogi have been known to exhibit health promoting properties such as control of gastroenteritis in animals and man. This has been demonstrated by locals who drink the raw slurry when there is a case of diarrhoea. In vitro and in vivo data had been obtained on the probiotic effects (hypolipidemic, hepatoproctective and anti-bacterial) of some lactic acid bacteria isolated from ogi [11,12].

However, studies relating to the antibacterial properties of these organisms have been limited and not fully exploited for use [13,14]. Foods can be supplemented with ex situ produced bacteriocin preparations, or by inoculation with the bacteriocin-producer strain under conditions that favour production of the bacteriocin *in situ* [15,16].

Ogi is a popular and readily available fermented food material in Nigeria, and bacteriocins from lactic acid bacteria have been proven to be effective against most enteric bacteria. This study was therefore done to determine the effects of pH and storage temperatures on the antibacterial activity of the bacteriocin produced by lactic acid bacteria isolates from Ogi.

2. MATERIAL AND METHODS

2.1 Sample Collection

Ogi sample used for the study was purchased from local vendors who hawk the product around the Benson Idahosa University campus.

2.2 Isolation of Lactic Acid Bacteria from the Ogi Samples

The Lactic acid bacteria of ogi was isolated using MRS1 agar (MRS with 0.1% glucose, and 50 µg/ml of cycloheximide) and M17 m agar (0.5% glucose and 50 µg/ml of cycloheximide) and thereafter characterised using standard biochemical tests [17,18].

2.3 Screening of Lactic Acid Bacteria for Antibacterial Activity

Indicator organisms used were *Escherichia coli* and *Staphylococcus aureus.* Antimicrobial activity of bacterial isolates was screened by the agar-well diffusion method using the cell free supernatants [19].

2.4 Production of Crude Bacteriocin

Lactobacillus plantarum which had produced the highest zone of inhibition during the screening test was utilized in the production of crude bacteriocin. *L. plantarum* was propagated in sterilized 500 ml MRS broth(pH 7.0, glucose 0.25% w/v) incubated for 72 h at 30ºC under anaerobic conditions [20]. For extraction of bacteriocin, a cell-free solution was obtained by centrifuging cultures at 10,000 rpm for 20 min. The culture was adjusted to pH 7.0 by means of 1M NaOH which also serve to exclude the antimicrobial effect of organic acid, followed by filtration of the supernatant through Whatman no.1 filter paper [20]. Inhibitory activity from hydrogen peroxide was eliminated by the addition of 5 mg/ml catalase [21].

2.5 Determination of Bacteriocin Activity

Ten ml of crude bacteriocin extract was serially diluted up to 10^{-2} using saline solution as diluent. Aliquots of 50 µl from each dilution were placed in wells (8 mm diameter) in plates seeded with overnight broth cultures of *E. coli* and *S. aureus*. Also, the pH of the diluted crude bacteriocin extract was also determined with the aid of a calibrated Jenway pH meter (Adventurer Ohaus Company, USA). These plates were incubated anaerobically at 30ºC for 24h. The incubated plates examined for the presence of clear zones of inhibition around the wells and the zones were measured in mm.

2.6 Determination of the Effects of pH on the Activity of the Crude Bacteriocin Extract

Ten ml of the crude bacteriocin extract was serially diluted up to 10^{-2} using saline solution as diluent. The content of diluted extract and the stock extract was divided into five equal portions. To each of the respective portions, drops of 1M HCL and 1M NaOH was added to give extracts with different pH of 2, 4, 6, 8 and 10 respectively [22]. The final pH of the treated diluted extract was confirmed with the aid of calibrated Jenway pH meter (Adventurer Ohaus Company, USA). Also, aliquots of 50 µl from each treated diluents were placed in wells (8 mm diameter) in plates seeded with overnight broth cultures of *E. coli* and *S. aureus*. These plates were incubated anaerobically at 30ºC for 24 h. The incubated plates were then examined for the presence of clear zones of inhibition around the wells and the zones measured in mm.

2.7 Determination of the Effects of Storage Conditions on the Activity of the Crude Bacteriocin Extract

Ten ml of the crude bacteriocin extract was serially diluted up to 10^{-2} using saline solution as diluent. The content of diluted extract and the stock extract was divided into three equal portions. Each of these respective portions was kept at several temperatures for 7 days:- ambient room temperature (28±2ºC), -2ºC and -20ºC [22]. At the end of the storage period, the effects of the respective storage temperatures on the antibacterial activity of the bacteriocin extracts were evaluated using the agar well diffusion technique. The indicator organisms utilized were clinical isolates of *E. coli* and *S. aureus* gotten from the microbiology laboratory of the University of Benin Teaching hospital. The isolates were re-identified using standard biochemical tests (18). The plates were incubated at 30ºC for 24h under anaerobic conditions. After incubation, the respective plates were examined for the presence of clear zones of inhibition around the wells and the zones were measured in mm.

3. RESULTS

List of lactic acid bacteria isolated from the study is presented in Table 1. The result shows that *Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus brevis, Lactobacillus casei,* and *Lactobacillus jensenii* were the lactic acid bacteria isolated.

The highest zones of inhibition (Fig. 1) were exhibited by *Lactobacillus plantarum*. However the lowest zones of inhibition were produced by *Lb. jensenii* against *E. coli* and *S. Aureus*

Serially diluted crude bacteriocin extract (10^{-1}) produced the highest zone of inhibition against *E. coli*, whilst the diluted extract (10^{-2}) gave the lowest zone of inhibition against *S. aureus* (Fig. 2).

Serially diluted crude bacteriocin extract (10^{-1}) at pH 2 produced the highest zone of inhibition against *S. aureus*. The result shows the same zone of inhibition (6 mm) for *S. aureus* at pH 6 and 8 and then reduced to 2 mm at pH 8. The lowest zone of inhibition was produced against *E. coli* at pH 6 and pH 8. At pH 10 there was no zone of inhibition for both organisms (Fig. 3).

The serially diluted crude bacteriocin extract (10^{-2}) produced a high zone of inhibition (11 mm) against *S. aureus* and the low zone of inhibition (1mm) against *E. coli* at pH 2 and 8. At pH 6, there was no zone of inhibition against *E. coli*, also at pH 10; there was no zone of inhibition against both *E. coli* and *S. aureus* There was however a zone of inhibition of 2mm against *E. coli*, at pH 4 (Fig. 4).

Table 1. List of lactic acid bacterial isolates from Ogi

Samples	Lactic acid Bacterial Isolates
Ogi Sample	*Lactobacillus plantarum* *Lactobacillus fermentum* *Lactobacillus brevis* *Lactobacillus casei* *Lactobacillus jensenii*

The serially diluted crude bacteriocin extract (10^{-1}) stored at -20ºC (Fig. 5) produced the highest zone of inhibition against *E. coli*, while the serially diluted crude extract (10^{-1}) stored at $28±2^{c}$ also produced the lowest zone of inhibition against *E. coli*. However it had the highest activity against *S. aureus* at 28±2ºC and lowest at 4ºC.

Serially diluted crude bacteriocin extract (10^{-2}) stored at -20ºC produced the highest zone of inhibition against *E. coli*, while the serially diluted crude extract (10^{-2}) stored at 4ºC produced the lowest zone of inhibition against *E. coli*. Crude extract stored at 28±2ºC produced no zone of inhibition against *E. coli.*

Result for *S. aureus* was however different from that observed for *E. coli*. Though the zone of inhibition was lowest at 4ºC, it was the same at both 28±2ºC and -20ºC.

4. DISCUSSION

The isolation of the respective Lactobacilli especially *L. plantarum*, *L. fermentum*, *L. brevis, L. casei and L jensenii* (Table 1) from ogi samples, was not surprising, as the presence of these respective Lactobacilli has been reported in ogi fermentation [23,24,25,26,27,9]. Amongst the several *Lactobacillus* spp. isolated from ogi, *L. plantarum* exhibited the highest antimicrobial activity against *E. coli* and *S. aureus* during the screening test (Fig. 1). This observation is similar to those reported by [25] who indicated that 64.29% of *L. plantarum* isolates were active producers of extracellular antimicrobial polysaccharides.

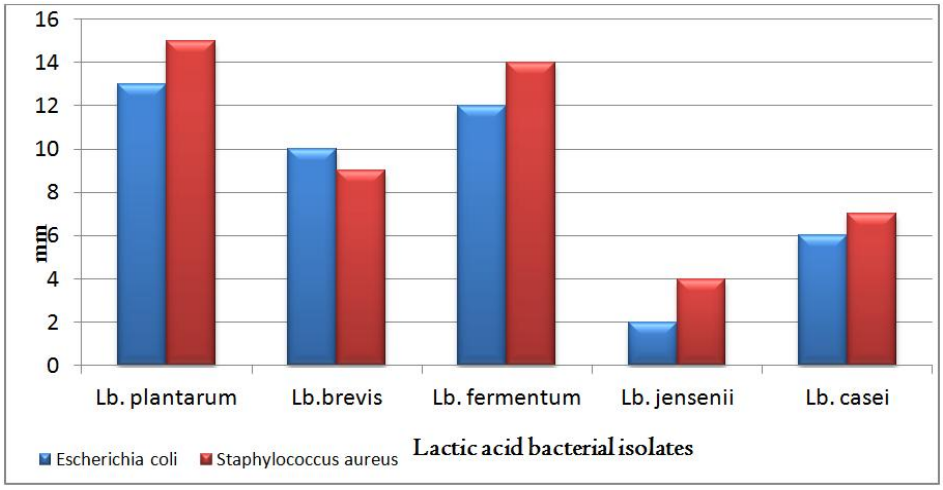

Fig. 1. Screening of the lactic acid bacterial isolates for antimicrobial activity

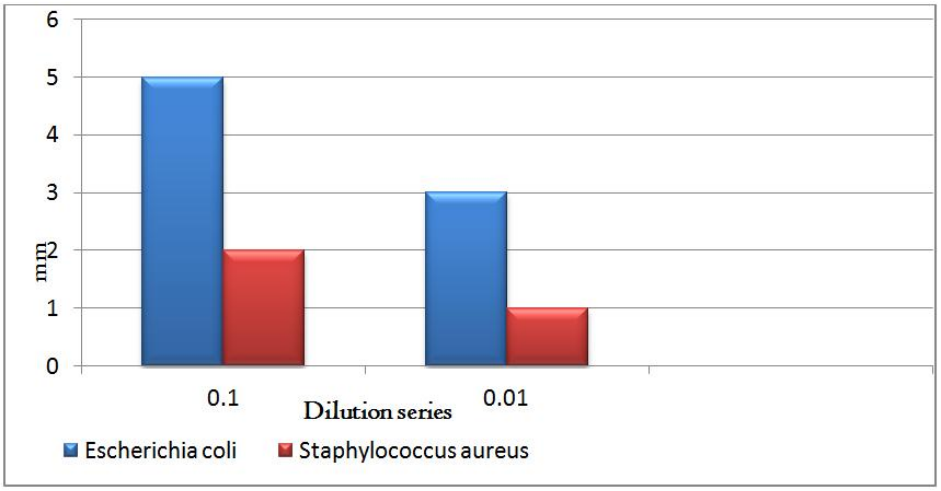

Fig. 2. Determination of the antimicrobial activity of the diluted crude bacteriocin extract

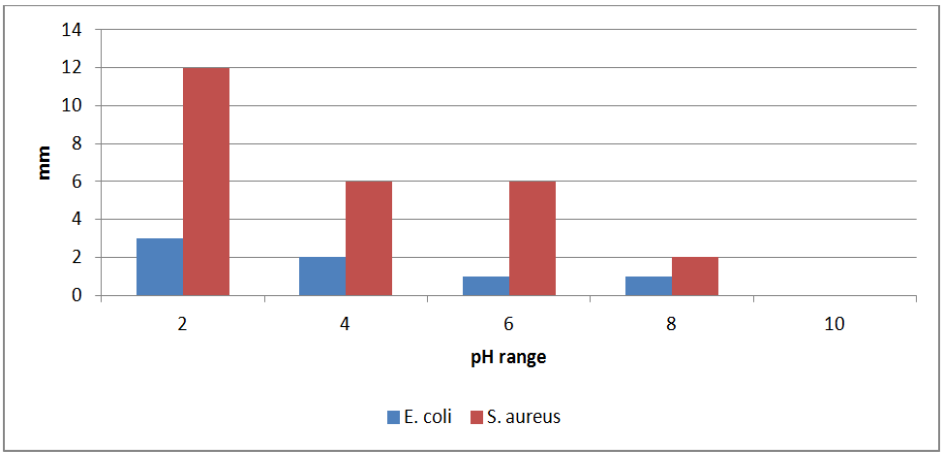

Fig. 3. Bacteriocin activity of the serially diluted crude bacteriocin extract (10^{-1}) against indicator organisms at different pH

The effect of dilution was noticeable on the antimicrobial activity of the crude bacteriocin extract recovered from *L. plantarum,* as the diluted extract (10^{-1}) exhibited an inhibition zone for *E. coli* (5 mm) as against a zone of inhibition (2 mm) for *E. coli* displayed by the successive diluted extract (10^{-2}) (Fig. 2). The diluted crude bacteriocin extract recovered from this isolate displayed a very high antimicrobial activity at pH 2 compared to the antimicrobial activity displayed at pH 4, 6 and 8 (Figs. 3 and 4). No antimicrobial activity was displayed by the serially diluted extract at pH 10. This observation could be due to the fact that the producer organism (*L. plantarum*) has a high tolerance to low pH [28]. This observation is similar to that reported by [20] which indicated that purified bacteriocin extract recovered from *L. plantarum* was more active at pH 2 and 6, than at pH 10 and 12. The increased sensitivity of *S. aureus* to the pH amended crude bacteriocin extract could be as a result of the cell wall and membrane physiology of the bacterium. This observation is in agreement with a report by [29] who observed that the antibacterial activity of purified bacteriocin extracted from *L. paracasei* subsp. *tolerans* was more active against *S. aureus* and *Listeria monocytogenes* than against *E. coli.* The diluted crude bacteriocin extract at pH 10 exhibited no antimicrobial activity against the indicator organisms. This could indicate that high pH value had a negative effect on the antibacterial activity of the extract.

The bacteriocin extract exhibited maximal antibacterial activity against *E. coli* when stored at -20ºC for 7 days (Figs. 5 and 6), whilst at ambient temperature (28±2ºC) the extract exhibited minimal antibacterial activity. Interestingly, the extracts exhibited the same antibacterial activity against *S. aureus* when stored at -20ºC for 7 and also at ambient temperature. The activity was reduced at 4^0C. This observation which is different from that recorded for *E. coli* is an interesting one judging from the fact that *S. aureus* is a gram positive organism while *E. coli* is a gram negative organism. This is not to suggest that it is same for all gram positives and gram negatives. Further studies will need to be done to confirm this pattern and plausible reasons suggested for the observation. This apparent expression of antibacterial activity at this storage temperature (-20ºC) could suggest its stability at this temperature. This observation could also reflect that cold storage at this temperature may be the most appropriate preservation method for the extract against *E. coli*. Ogunbanwo et al. [20] recorded similar results for purified bacteriocins recovered from *L. plantarum* and *L. brevis* which retained their stability when stored for 60 days at -20ºC.

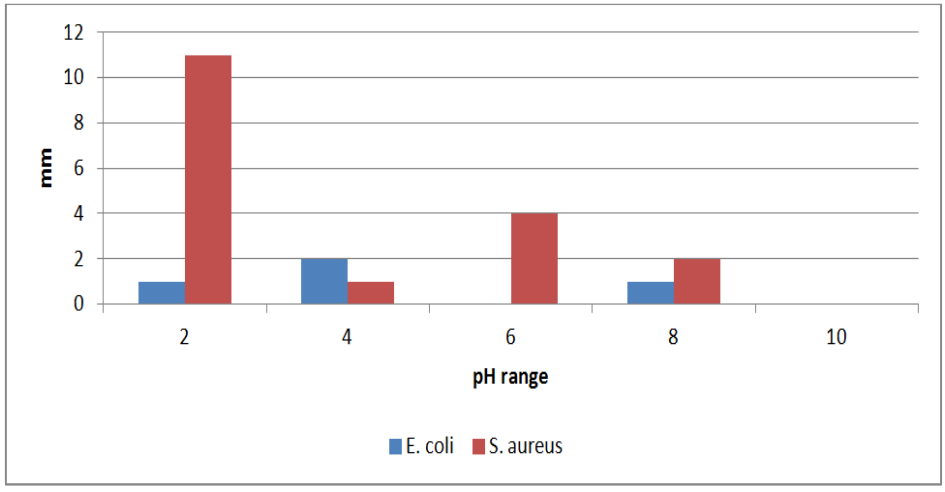

Fig. 4. Bacteriocin activity of the serially diluted crude bacteriocin extract (10^{-2}) against indicator organisms at different pH

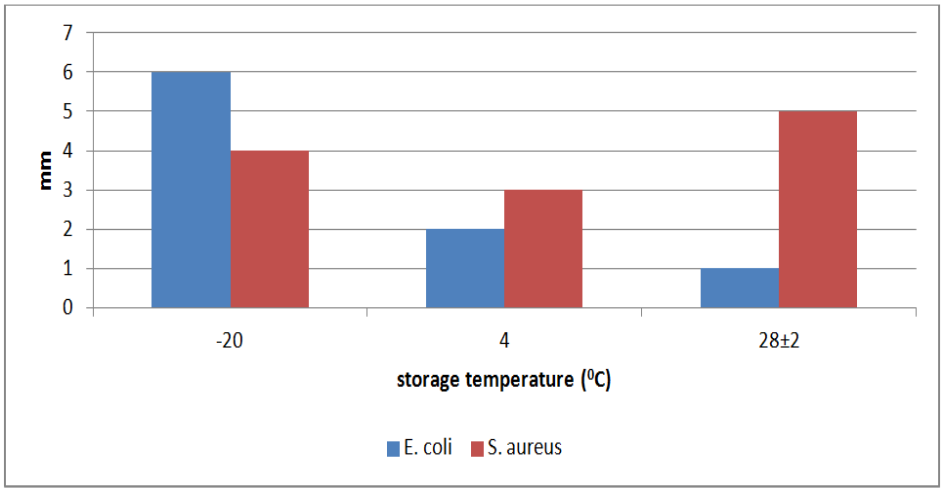

Fig. 5. Bacteriocin activity of the serially diluted crude bacteriocin extract (10^{-1}) against indicator organisms at different storage temperatures

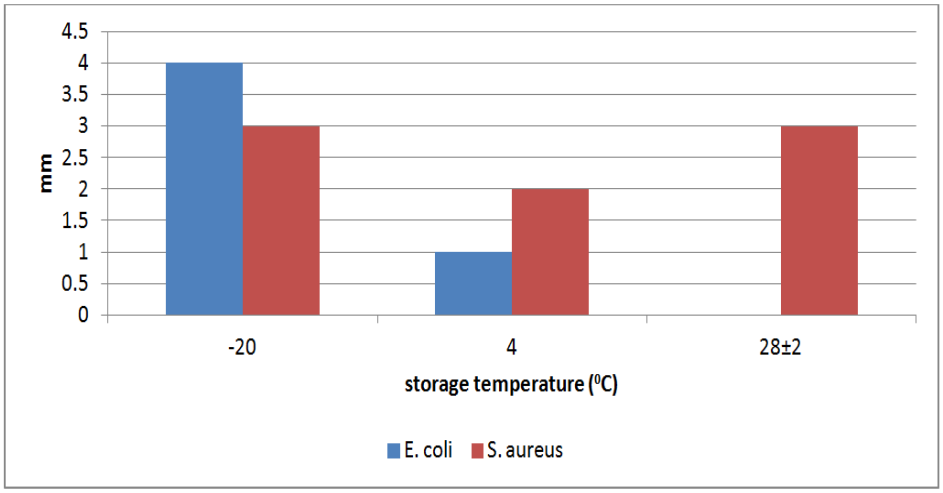

Fig. 6. Bacteriocin activity of the serially diluted crude bacteriocin extract (10^{-2}) against indicator organisms at different storage temperatures

5. CONCLUSION

Ogi is a viable source of several Lactobacilli which are capable of producing several antimicrobial compounds such as bacteriocins. It is recommended that the crude bacteriocin extract recovered from *L. plantarum* should be further purified and its potential antimicrobial activities against a variety of both diarrhoegenic bacterial isolates and spoilage microorganisms investigated. This could improve the hygiene and safety of the food products so produced.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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