ENHANCED BACTERIOCIN PRODUCTION THROUGH CULTURAL SUPPLEMENTATION USING *Leuconostocmesenteriodes* FCF23 AS PRODUCER ORGANISM

*[1]**Mohammed** Sani Sambo Datsugwai, [2]**Damisa** Duro, [2]**Abdulrahman** AlhassanAbdullahi,
[3]**Ayansina** Ayantade Dayo Victor
*[1]Ibrahim BadamasiBabangida University,Department of Microbiology,Lapai, Niger Stare, Nigeria.
[2]Federal University of Technology,Department of Microbiology, Minna, Niger State. Nigeria.
[3]Bowen University Department of Biological Sciences,,Iwo, Osun State. Nigeria.
* Email: mosada78 @yahoo.

Astract
Cultural supplementation on bacteriocin production by Leuconostocmesenteroides FCF23 was investigated. The cultural supplements of De Man Rogosa Sharpe (M.R.S) broth demonstrated that large quantities of bacteriocins was produced only when the addition of 1.0,2.0 and 3.0% glucose, 2.0% and 3.0% of NaCl,1.0,2.0 and 3.0% of beef extract, 1.0,2.0 and 3.0% (w/v)of yeast extract, 1.0% (v/v) Tween 80 and 1.0% (w/v) triammonium citrate with improved bacteriocin activity (AU/mL) from 7800 AU/mL to 8000 AU/mL while addition of (w/v) 0.5 and 1.0% of sodium acetate, magnesium sulphate, manganese sulphate, and dipotassium hydrogen phosphate had decreasing effects on enhanced bacteriocin production. The bacteriocins produced by test isolates inhibited the growth of spoilage pathogenic microorganisms (Escherichia coli N2,Pseudomonas aeruginosa N7,Listeria monocytogenes W6,Shigella dysenteriae N11, Bacillus cereus W18,Salmonella typhimurium N8, Escherichia coli W4,Proteus vulgaris W7,Klebsiella aerogenes N12,Staphylococcus aureus N16b,Bacillus subtilis N20,Klebsiella ozaenae W24,Proteus mirabilis N16a. L.mesenteroides FCF23) and had the bacteriocin activity of 8000±00.0 AU/mL. Enhanced bacteriocin production was achieved at pH between 4.00 and 4.30 and incubation period of 48 hours at temperature of 30°C by L. mesenteroides FCF23 used as producer organism. Therefore the variation in the concentration of constituents/supplements of cultivating media (MRS broth) have an influence on the amount of bacteriocin produced by Leuconostocmesenteroides FCF23The lactic acid bacteria (LAB) play very important role in food safety and bio-preservation due to the metabolites (eg.bacteriocins) they produced during stress and when present in food products at favorable conditions. Bacteriocins are therefore useful in Biopreservation processes in food and dairy industries.

Keywords: Bacteriocins, *Leuconostocmesenteroides*, De Man Rogosa Sharpe broth, spoilage pathogenic microorganisms, Cultural supplementation.

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1.INTRODUCTION

Lactic acid bacteria (LAB) are a group of Gram-positive bacteria, non-spore forming, non-respiring, cocci or rods, which produce lactic acid as the major end product of the fermentation of carbohydrate (Axelson, 1998).LAB is often inhibitory to other microorganisms and this is the basis of their ability to affect the keeping quality and safety of many food products. The low pH, organic acids hydrogen peroxide, ethanol, nutrient depletion, low redox potential are the principal factors which contribute to this inhibition and bacteriocin production (Adams and Nicolaides, 1997).

Bacteriocins are naturally occurring antibiotic peptides produced by Gram-positive bacteria, they are usually small, such as 24 amino acids (Chatterjee *et al*., 2005).Devugst and vanclamme (1994) worked on production of bacteriocin (nisin) using a specie of lactic acid bacteria (*Lactococcuslactis*Subsplactis) and reported that nisin was the first bacteriocin used on commercial scale as food preservative date back to the first half of the century.Though several types of bacteriocins from food-associated lactic acid bacteria have been identified and characterized, of which the important ones are diplococcin, acidophilin, bulgarican, helveticin, lactacin, plantaricin and nisin (Nettles and Barefoot, 1993).Vignolo*et*

al.(1995) revealed that enhanced or optimized bacteriocin production could be achieved by supplementing a culture medium with growth limiting factors. Such as Vitamins, Sugars and Nitrogen Sources, by choosing the best-adapted culture medium or by regulating the culture pH.

Fermented corn flour or starch (also called Akamu, Ogi) is prepared by first cleaning the cereal grain (*zea mays*) and steep in water for 2 days in earthenware pot (or any suitable container). The water is decanted and the grains wet-milled before sieving with muslin cloth or a fine wire mesh. The pomace is then discarded and the starch suspension is allowed to sediment during which fermentation is carried out for 2-3 days by the natural flora of the grains (Odunfa, 1985). Ogunbawo*et al.* (2003) isolated lactic acid bacteria from Ogi. The LAB isolated include *Lactobacillus fermentum, L. casei, L. plantarum, L. brevis and L. reuteri*for bacteriocin production. For the benefit of this study, the significance of the cultural supplementation on bacteriocin production by *Leuconostocmesenteroides* FCF23 is the sole aim of this research

2. MATERIALS AND METHODS

Collection of samples

Fermented corn flour ('akamu' or 'ogi'),Nono and Wara were collected separately in clean sample bottles from Bosso Market and was transferred to the Laboratory for the Isolation of Lactic bacteria (LAB) and Spoilage Pathogenic Microorganisms.

Isolation Lactic Acid Bacteria and Spoilage Pathogenic Microorganisms

The pour plate methods of Fawole and Oso. (1998).,Cheesbrough. (2003).,Oyeleke and Manga. (2008) were followed for the isolation of LAB and spoilage pathogenic microorganisms from the fermented corn flour,nono and wara respectively.

Culture Media

The culture media used in this research were prepared following the standard laboratory methods as prescribed by Daba*et al*.(1991) and Cheesebrough (2003). The media used in this study include Nutrient agar (NA) (Oxoid), Urea agar base (Analar), Mannitol salt agar (MSA) (Oxoid), Simon's citrate agar (Oxoid), De Man Rogosasharpe (MRS) broth (Oxoid) and Lactic Acid Medium (LAM) (Oxoid). Lactic acid medium (LAM) is a selective medium for the growth of lactic acid bacteria.

Characterization of and Identification of Microbial Isolates

Microbial isolate**s** were characterized and identified based on colony morphology,cell morphology and biochemical tests described by Fawole and Oso. (1998).,Cheesbrough. (2003).,Oyeleke and Manga (2008).The LAB was identified as *Leuconostocmesenteroides* FCF23 using the scheme of Cheesbrough (2003).

Cultural Supplementation to Enhanced Bacteriocin Production

The cultural supplements on bacteriocin production by *Leuconostocmesenteroides* FCF23 was evaluated according to the procedure of Ogunbanwo*et al*. (2003) by incorporating each of the following salts in to De Man Rogosa Sharpe (MRS) Medium. Glucose (1.0,2.0 and 3.0%), NaCl (1.0,2.0 and 3.0%), beef extract (1.0,2.0 and 3.0%), yeast extract (1.0,2.0 and 3.0%), Tween 80 (0.5 and 1.0%), Triammonium citrate (0.5 and 1.0%), $MnSO_4$.$4H_2O$ (0.5 and 1.0%), K_2HPO_4 (0.5 and 1.0%), Sodium acetate (0.5 and 1.0%), and $MnSO_4$. $7H_2O$ (0.5 and 1.0%). The broth culture was tested for bacteriocin activity, pH and growth (nm) (Rammelsberg and Radler, 1990 brinkten*et al*, 994 Balasubramanian and Varadaraj, 1998).

Bacteriocin Production after Cultural Supplementation

Leuconostocmesenteroides FCF23 was propagated in 1000ml of De Man Rogosa Sharpe (MRS) broth supplemented with the nutrients (pH 5.8) at 30^{o}C for 48 hours for each of the isolates respectively.

Extraction of Cell Free Bacteriocins

For extraction of bacteriocins,a cell free solution was obtained by centrifugation

(10,000 rpm for 20 minutes).The culture was then adjusted to pH 7.0 using 1M NaOH to exclude the antimicrobial effect of organic acid, then followed by filtration of the supernatant through 0.2µm pore size cellulose acetate filter to obtain crude bacteriocin (Schillinger and Lucke, 1989).

Elimination of Inhibitory Activity

Inhibition activity from hydrogen perioxide (H_2O_2) was eliminated by the addition of 5 mg/ml catalase (*Daba et al*., 1991).

Testing of Bacteriocin Broth Culture

The broth culture was tested for pH, bacteriocin activity and growth (nm) (Brinkten *et al.,* 1994., Graciela *et al*., 1995). The broth culture obtained was tested for pH, (*Escherichia coli* N2,*Pseudomonas aeruginosa* N7,*Listeria monocytogenes*W6,*Shigella dysenteriae*N11, *Bacillus cereus* W18,*Salmonella typhimurium*N8, *Escherichia coli* W4,*Proteus vulgaris* W7,*Klebsiella aerogenes*N12,*Staphylococcus aureus* N16b,*Bacillus subtilis* N20,*Klebsiella ozaenae*W24,*Proteus mirabilis* N16a) has described by Brinkten*et al*. (1994), Graciela *et al*. (1995) and Ogunbanwo*et al*.(2003).

3. RESULTS AND DISCUSSION

3.1 RESULTS

Leuconostocmesenteroides FCF23 was observed to be good bacteriocin producer after propagating in De Man Rogosa Sharpe broth supplemented with some nutrients at different concentrations. The effects of nutrient supplements on bacteriocin production by *L. mesenteroides* FCF23 showed that large amount of bacteriocin was obtained only when the MRS medium was supplemented with 1.0,2.0 and 3.0% glucose, 2.0% and 3.0% of NaCl,1.0,2.0 and 3.0% of beef extract, 1.0,2.0 and 3.0% (w/v)of yeast extract, 1.0% (v/v) Tween 80 and 1.0% (w/v) triammonium citrate with improved bacteriocin activity (AU/mL) from 7800 AU/mL to 8000 AU/mL while addition of (w/v) 0.5-1.0% of sodium acetate, magnesium sulphate, manganese sulphate, and dipotassium hydrogen phosphate had decreasing effects on enhanced bacteriocin production (Table 1).*L. mesenteroides* FCF23 had producing ability of between 0.88 to 0.94, pH of 4.00 to 4.30 and bacteriocin activity (AU/mL) of 7800 to 8000 (Table 1).The inhibition of *Escherichia coli* N2,*Pseudomonas aeruginosa* N7, *Listeria monocytogenes*W6, *Shigella dysenteriae* N11, *Bacillus cereus* W18, *Salmonella typhimurium*N8, *Escherichia coli* W4, *Proteus vulgaris* W7, *Klebsiella aerogenes*N12, *Staphylococcus aureus* N16b, *Bacillus subtilis* N20, *Klebsiella ozaenae*W24 and *Proteus mirabilis* N16a was recorded as +8mm for each spoilage pathogenic microorganism respectively (Table 2).

3.2 DISCUSSION

The variation in the concentration of constituents/supplements of cultivating media (MRS broth) have an influence on the amount of bacteriocin produced by *Leuconostocmesenteroides*FCF23. The highest bacteriocin activity of 8000 AU/mL was exhibited by *Leuconostocmesenteroides*FCF23 when 1, 2, and 3% Glucose, 2 and 3% NaCl, 1,2 and 3% beef extract,1, 2 and 3% yeast extract and 1% tween 80 was added to MRS broth. This is similar to the findings of Mohammed *et al.*(2013) who revealed that highest bacteriocin production was achieved when 2%. Glucose, 1% NaCl, 2% beef extract,1, 2 and 3% yeast extract and 1% tween 80 was added to MRS broth. While *L lactis* N22 exhibited highest bacteriocin activity of 5800±0.00 AU/mL when 2% beef extract, 1, 2 and 3% yeast extract, 0.5 and 1% tween 80 were added to MRS broth. While the supplements of triammonium citrate, manganese sulphate, potassium hydrogen sulphate, sodium acetate and magnesium sulphate at 0.1% and 0.5% yield no improvement in bacteriocin production.. Similar observations have been made previously by Sani *et al*. (1999) who reported that when glucose and peptone were varied to 0.25% and 0.5% in the constitute MRS broth, high bacteriocin activity was recorded. While bacteriocin activity was not detected at 2% glucose and peptone level. The ineffectiveness of manganese sulphate, potassium hydrogen

sulphate, sodium acetate and magnesium sulphate supplemented with the MRS broth might be due to the activity of extracellular endogenous proteinases induced during the growth phase of the test strains.This is in conformity with the findings of Paired*et al.* (1990) who revealed that after nutrient supplementation, the decrease in bacteriocin production by *Lactococcuslactis* CNRZ 481 could be due to activity of extracellular proteinases induced during the growth phase.Some reports indicate that bacteriocin are produced throughout the experimental growth phase and note solely during late logarithmic or early stationary phase (Joerger and Klaenhammer, 1986., Paid *et al.,* 1990).The bacteriocin produce by *L mesenteroides*FCF23 exhibited bacteriocin activity between 1000 to 8000 AU/mL against *Escherichia coli* N2, *Pseudomonas aeruginosa* N7, *Listeria monocytogenes*W6, *Shigella dysenteriae*N11, *Bacillus cereus* W18,*Salmonella typhimurium*N8, *Escherichia coli* W4, *Proteus vulgaris* W7, *Klebsiella aerogenes*N12, *Staphylococcus aureus* N16b, *Bacillus subtilis* N20, *Klebsiella ozaenae*W24 and *Proteus mirabilis* N16a.

Table 1 .Cultural supplementation on bacteriocin production by *Leuconostocmesenteroides*FCF23.

Medium composition	Quantity of nutrient used (%)	Growth (580nm)	pH of medium	Bacteriocin activity (AU/ml)
MRS (Unsupplemented)	0.0	0.88	4.00	7800
MRS + glucose	1.0	0.89	4.10	8000*
	2.0	090	4.10	8000*
	3.0	0.91	4.10	8000*
MRS + NaCl	1.0	0.90	4.01	7800
	2.0	0.91	4.20	8000*
	3.0	0.93	4.30	8000*
MRS + Beef extract	1.0	0.91	4.20	8000*
	2.0	0.92	4.20	8000*
	3.0	0.93	4.30	8000*
MRS + yeast extract	1.0	0.90	4.20	8000*
	2.0	0.92	4.20	8000*
	3.0	0.94	4.20	8000*
MRS + Tween 80	0.5	0.90	4.00	7800
($C_{64}H_{124}0_{26}$)	1.0	0.93	4.22	8000*
MRS + Triamonium citrate	0.5	0.90	4.00	7800
($CH_2C0HCH_2(C00^-NH_4^+)_3$)	1.0	0.92	4.20	8000*
MRS + $MnS0_4 \cdot 4H_20$	0.5	0.42	2.40	4000
	1.0	0.30	1.50	2000
MRS + K_2HP0_4	0.5	0.40	2.50	4000
	1.0	0.20	1.00	1000
MRS + Sodium acetate	0.5	0.20	1.50	1000
(CH_300^-Na)	1.0	0.30	1.40	1000
MRS + $MgS0_4 \cdot 7H_20$	0.5	0.31	2.42	2000
	1.0	0.21	1.00	1000

Growth, pH and bacteriocin determinations were done after 48 hours of incubation ,MRS: De Man Rogosa medium;AU/mL: Activity Units per mililitre; nm: nanometer,*:enhanced bacteriocin produced.

Table 2: Inhibition of spoilage pathogenic microorganisms by bacteriocins produced by *Leuconostocmesenteroides*FCF23after nutrient Supplements of MRS.

Spoilage pathogenic microorganisms	Source	Zone of inhibition (mm) caused by bacteriocins produced by *Leuconostocmesenteroides*FCF23.
E.coli N2	N	+8
P.aeruginosa N7	N	+8
L.momocytogenes W6	W	+8
S.dysentriae N11	N	+8
B.cereus W18	W	+8
S.typhimurium N8	N	+8
E.coli W4	W	+8
P.vulgaris W7	W	+8
K.aerogenes N12	N	+8
*S. aureus*N16b	N	+8
B.subtilis N20	N	+8
K.ozaenae W24	W	+8
P.mirabilis N16a	N	+8

N: Nono,W:Wara,mm:millimeter,+:Inhibition

This is in conformity with the findings of Ogumbanwo*et al*. (2003) who reported that *Listeriamonocytogene*, *Escherichia coli* and *Enterococcusfaecalis* were inhibited by the bacteriocin produced by *Lactobacillus plantarium* F1, and *Lactobacillus brevis* OG1 with bacteriocin activity between 3200±0.00 to 6400±0.00 Au/mL.This findings also agrees with Mohammed *et al*.(2012) who reported that pediocin and nisin produced by *Pediococcushalophilus*W9 and *Lactococcuslactis* N24 exhibited bacteriocin activity between 5000±0.00 and 5600±0.00 AU/mL against *Pseudomonas aeruginosa, Shigelladysenteriae* and *Eschericiacoli.* Similarly,Biwas*et al*. (1991) reported that supplementations of culture nutrient of cultivating media should be considered for enhanced production of bacteriocin that has potential use as biopreservative

4.CONCLUSION

In conclusion, bacteriocin could be largely produced if the cultural medium is supplemented with nutrients that could favor the growth and comfort ability of the producer organisms (LAB).This will stimulate the LAB lyses' gene to release large amount of bacteriocin from it producing cells.

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6. RECOMMENDATION

It is therefore recommended that the enhanced bacteriocin produced be employed in food and dairy industries for Biopreservation purposes/processes.

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