

Full Length Research Paper

Bacteriocin and cellulose production by lactic acid bacteria isolated from West African soft cheese

Adetunji, V.O.^{1*} and G.O Adegoke²

¹Department of Veterinary Public Health and Preventive Medicine University of Ibadan, Ibadan, Nigeria.

²Department of Food Technology, University of Ibadan, Ibadan, Nigeria.

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Sixteen colonies of lactic acid bacteria (LAB) were selected and screened for their ability to produce bacteriocin by agar well diffusion method using the supernatant of centrifuged test cultures. Four isolates inhibited the growth of *Listeria monocytogenes* and *Escherichia coli*. *Lactobacillus plantarum* (6) and *Lactobacillus brevis* (5) were the most dominant species. The remaining were *Lactobacillus lactis* (2), *Streptococcus lactis* (2) and *Lactobacillus fermentum* (1). *Lactobacillus* spp. accounted for 87.5% of all isolates. LAB4 (*Lactobacillus plantarum*) showed some levels of antimicrobial activity after 15, 20 and 25 min heat treatments at 100°C against *Listeria monocytogenes*. While antimicrobial activity of LAB70 (*Lactobacillus lactis*) was against both *Listeria monocytogenes* (after 20 and 25 min) and *E. coli* 0157:H7 (after 15, 20 and 25 min) heat treatment at 100°C. All the lactic acid bacteria used in this study produced cellulose. The correlation between cellulose production (an adhesion factor) and bacteria growth was highly significant after 72 h of incubation having a $R^2 = 0.800$. This study offers useful information on growth and cellulose production as factors affecting the efficacy of bacteriocin produced by these strains which could be good for biopreservation.

Key words: Lactic acid bacteria, antimicrobial, bacteriocin, cellulose, growth.

INTRODUCTION

Lactic acid bacteria (LAB) and physiologically related group of gram-positive bacteria produce a variety of compounds with antimicrobial activity, and they are termed bacteriocins. Bacteriocins are generally defined as extracellularly released peptide or protein that shows a bacteriocidal activity against species closely related to the bacteriocin producing strain. Many bacteriocins show broader spectrum of activity against more distantly related species like *Listeria monocytogenes* (Adam and Moss, 1995).

The interest in bacteriocins produced by LAB has grown because many bacteriocins inhibit food spoilage and pathogenic bacteria such as *L. monocytogenes* which are recalcitrant to traditional food preservation method (Cleveland et al., 2001). In addition, bacteriocinogenic LAB are associated with and are used as starter cultures (Cleveland et al., 2001). The use of bacteriocin

or bacteriocin producing organism in the food industry is attractive because there is an increasing demand for natural products and increasing concern about foodborne diseases (Cleveland et al., 2001).

Lactic acid bacteria and their metabolites have been shown to play an important role in improving microbiological quality and shelf life of many fermented food products and provide a good example of biopreservation (Zottola et al., 1994). Lactic acid bacteria specifically *Lactococcus lactis* produce nisin, a bacteriocin that has received particular attention because of its large inhibitory effect against a wide variety of gram-positive organisms (Rodriguez, 1996). Nisin is a polypeptide containing 34 amino acids and it is remarkably heat stable at acid pH (Adam and Moss, 1995). Nisin is a hydrophobic compound which can be degraded by metabisulphite, titanium oxide, and certain, proteolytic enzymes (Adam and Moss, 1995). Among some of its desirable properties as a food preservative are: nontoxicity, produced naturally by *L. lactis* strains, heat stable and has excellent storage stability, destroyed by digestive enzymes, does not contribute to off-flavours or off-odours, and has a narrow

*Corresponding author. E-mail: vadetunji@gmail.com.

spectrum of antimicrobial activity (Jay, 2000). Nisin is the only bacteriocin generally recognized as safe (GRAS) and the World Health Organization has approved its use as food additive (Hurst, 1981; Holzapfel et al., 1995). Higher acid values reported for nisin-containing cheese may be due to autolysis of nisin sensitive sub-populations of selected *Lactococci* and *Lactobacillus casei* and/or to the enzymatic compartment of the nisinogenic strain (Bouksaim et al., 1999).

Cellulose is an exopolysaccharide produced by microbial cultures and are involved in cell adhesion and biofilm formation. Lignin, hemicellulose and xylosans are other products from a microbial culture which can be extracted with acetic-nitric acid reagent. Cellulose remains dissolved in H₂SO₄ and is determined by anthrone reagent (Updegraff, 1969). Enhancement of cellulose production in a coculture with various *Lactobacillus mali* strains showed that cell-cell interaction assisted by exopolysaccharide producing *L. mali* promotes cellulose production in st-60-12 (cellulose producing acetic-acid bacterium belonging to *Glucacetobacter xylinus*) (Seto et al., 2006).

West African soft cheese is a soft, white unripened cheese in West Africa (Adegoke et al., 1992). West African soft cheese contained 70.5% moisture, 39.00% fat, 37.08% protein and 2.53% as reported by Alalade and Adeneye (2006). The most dramatic change which occurs in soft cheese after manufacture is the disappearance of lactose which especially with sour cheese was reduced from 4.6% to 0.2% within a period of 12 h after manufacture due to activities of lactic acid bacteria (Ogundiwin and Oke, 1983). This study reports on bacteriocin and cellulose production by lactic acid bacteria isolated from West African soft cheese.

MATERIALS AND METHOD

Preparation of culture

Lactic acid producing organisms were isolated from West African soft cheese on de man Rogosa agar and 16 colonies from plates were randomly selected. The selected colonies were then inoculated on de man Rogosa agar or M17 agar and incubated at 37°C. Microscopy and biochemical examinations were as described by Erdogan (2004).

Test for inhibitory quality of pure cultures of LAB species

The preliminary test for inhibitory quality of culture of LAB on pure cultures of *E. coli* (3 strains), and *L. monocytogenes* (5 strains) was carried out using agar diffusion method (Reinheimer et al., 1990).

Test for bacteriocin production

Test for bacteriocin production was done according to Aslim et al. (2005) with slight modification. 24 h fresh culture of LAB strains showing inhibitory qualities against test bacteria was grown on M17 agar and inoculated into tryptose soy broth and incubated at 37°C for 24 h. This was then incubated at 37°C for 24 h. The 24 h broth culture was then centrifuged at 3000 rpm for 5 min. The superna-

tant was decanted into sterile test tubes, adjusted to pH 6.5 - 7.0 with NaOH (40G/1000 ml) to remove organic acid effect. H₂O₂ was neutralized by addition of catalase from bovine liver at 200 µ/ml. The mixture of the supernatant of LAB culture, NaOH and catalase was filtered and sterilized with a 0.2 µm Millipore filter membrane. Inhibitory effect of free bacteriocin on test bacteria was then determined by agar well diffusion method using the filtrate from the mixture and the supernatant alone.

Mueller Hinton II agar plates were punched with 8 mm diameter sterile clubs. The bottom of each well was covered with viscous agar which was allowed to solidify to prevent spreading of the mixture and supernatant through the base. The wells were then filled with 20 µl of the mixture and filtrate of supernatant alone thereafter was refrigerated for 2 h to allow diffusion of test substrate, followed by incubation for 24 h at 37°C. The diameter of inhibition was then measured with meter ruler.

Heat sensitivity of bacteriocin

The bacteriocins produced were subjected to heat treatments at 100°C for 15, 20 and 25 min and tested according to the method of Aslim et al. (2005).

Quantification of cellulose produced by the LAB isolates

Quantification of cellulose production from LAB strains was done according to Updegraff (1969) method.

Assessment of bacterial growth using optical density

At each incubation period for the 10 ml culture of each LAB strain the culture was thoroughly vortexed and 1 ml of this culture was poured into a cuvette. The optical density was then taken at 620 nm wavelength against a reagent blank to give the bacterial growth in optical density (Capaldo-Kimpal and Barbour, 1971).

Statistical analysis

Experiments were done in two replicates and data subjected to analysis of variance of a complete randomized design model, multiple correlation and regression.

RESULTS AND DISCUSSION

Four out of 16 randomly picked colonies exhibited good antimicrobial activity against *E. coli* 0157: H7 (3 strains), *L. monocytogenes* (5 strains). All the LAB strains were gram positive, and all grew in 2% NaCl and none hydrolyzed starch. Based on criteria used by Erdogan and Sert (2004) identified strains include: *Lactobacillus plantarum* (6) and *Lactobacillus brevis* (5) were the most dominant species. The remaining were *L. lactis* (2) and *Streptococcus lactis* (2) and *Lactobacillus fermentum* (1). *Lactobacillus spp* accounted for 87.5% of all isolates.

One of the LABs isolated in this study (*L. lactis*) has also been found as one of the dominant flora of Beyaz cheese (Durlu-Ozkaya et al., 2001; Erdogan and Gurses, 2005). These reports are similar to what was obtained in this study. The different physiological reactions and sugar reduction also differentiates to species level.

Out of 16 strains of LAB which were screened, 4 strains

Table 1. Pattern of cellulose production measurement of bacteriocin producing LAB at 24, 48 and 72 h incubation periods.

Strains of LAB	Cellulose production at 24 h inc. (μg)	Cellulose production at 48 h inc. (μg)	Cellulose production at 72 h inc. (μg)	Growth (optical density) at 24 h Inc. (nm)	Growth (optical density) at 48 h inc. (nm)	Growth (optical density) at 72 h inc. (nm)
Control	0.300 ^a	0.300 ^a	0.300 ^a	0.1310 ^a	0.1310 ^a	0.1310 ^a
LAB4 ^{*D}	1.6445 ^a	1.4985 ^a	1.9065 ^a	1.0525 ^a	0.8580 ^a	1.2805 ^a
LAB3 ^{*A}	0.954 ^a	0.694 ^a	1.322 ^a	0.4625 ^a	0.2430 ^a	0.7440 ^a
LAB70 ^{*B}	1.6015 ^a	1.574 ^a	1.4785 ^a	0.9530 ^a	0.8435 ^a	0.8935 ^a
LAB42 ^{*C}	0.8475 ^a	0.786 ^a	0.7045 ^a	0.3655 ^a	0.258 ^a	0.2945 ^a

R² values = 0.800 at 72 h incubation; 0.200 at 48 h incubation; 0.350 at 24 h incubation.

LAB = Lactic acid bacteria; Inc. = incubation.

Lower case = Means with the same letter are not significantly different at $P < 0.05$ (variance)

Upper case = *Significant correlation at $P < 0.05$.

were found to show evidence of bacteriocin production with at least a minimum of 3 mm inhibition zone against the test organisms (*L. monocytogenes* and *E. coli* O157:H7) in the preliminary inhibitory test. LAB4 (*Lactobacillus plantarum*) showed some inhibitory activity after 15, 20 and 25 min heat treatments at 100°C against *L. monocytogenes* and LAB70 (*L. lactis*) inhibited both *L. monocytogenes* (after 20 and 25 min) and *E. coli* O157:H7 (after 15, 20 and 25 min) heat treatment at 100°C. Similar effect of *L. plantarum* has been observed earlier (Kandler and Weiss, 1986; Tarelli et al., 1994; Pal et al., 2005). Bacteriocin-like substances from 2 LAB strains (*L. lactis* and *L. plantarum*) were observed even after 25 min of heat treatment. A similar report was made by Aslim et al. (2005) who reported bacteriocin-like substances from lactic acid bacteria isolated from Turkish dairy products that were resistant to heat after 10 and 20 min at 100°C. The potential of a *Lactobacillus spp* to preserve vegetarian food system and a south Indian special dosa (appam) batter has been reported (Jamuna and Jeevaratnam, 2004; Jamuna et al., 2005). But the fact that not all the lactic acid bacteria strains in this study had inhibitory qualities against pathogenic bacteria showed that not all the strains produce antagonism effect. Jay (2000) also demonstrated that only some strains of lactic acid bacteria produce nisin. The use of bacteriocin in food preservation may not be a total remedy in heat treated foods since a reduction in viability was observed with heat treatment. Bacteriocin for food preservation should be heat stable as in nisin. The suppression of *L. monocytogenes* and *E. coli* is in agreement with an earlier report of Buyong et al. (1998), who genetically modified *L. lactis* to produce enough pediocin to control the growth of *L. monocytogenes*. All the lactic acid bacteria in this study produced cellulose. The correlation between cellulose production (an adhesion factor) and bacteria growth was highly significant by 72 h of incubation having a R² = 0.800 (Table 1). Significant correlations occurred between LAB strains 3, 4, 42 and 70 ($P < 0.05$), but there was no significant difference in variance ($P < 0.05$).

Extracellular polymeric substances of which cellulose a polysaccharide is one, are secreted during growth of microbes. The R² value being very close to 1 (0.800) at 72 h incubation can be explained by the fact that rate of cellulose production by lactic acid bacteria used in this study is indicative of cell growth although weak positive correlations were observed at 24 h (0.35) and 48 h (0.20) incubation. The LAB strains which produced the bacteriocin-like substances that were still inhibitory after 25 min heat treatment at 100°C had the faster growth rate and cellulose production at 24, 48 and 72 h incubation periods. This cellulose production which is a virulence factor in pathogenic organisms has been reported (Reed et al., 1988; Davies et al., 1993; Gulsun et al., 2005) and is probably the reason for the potency of the bacteriocin-like substances produced by *L. plantarum* and *L. lactis* strains in this study which is nonpathogenic.

Conclusion

Arising from this study, a positive correlation was observed between the efficacy of bacteriocin and cellulose production or bacteria growth. It is also possible that the 2 LAB (*L. plantarum* and *L. lactis*) isolates from West African soft cheese which were found to be capable of producing viable bacteriocin can be purified and characterized for use in biopreservation.

REFERENCES

- Adam MR, Moss RO (1995). Food Microbiology the Royal Society of Chemistry Thomas Garden House Science Park, Cambridge CB 40 wF 3rd Edition, pp. 156-218.
- Alalade OA, Adeneye JA (2006). The effect of storage period on the chemical composition and coliforms microflora of *wara* cheese. Int. J. Dairy Sci., 1: 126-130.
- Aslim B, Yuksekdogan ZN, Sarikaya E, Beyatli, Y (2005). Determination of the bacteriocin-like substances produced by some lactic acid bacteria isolated from Turkish dairy products. Lebensm. Wiss. Technol., 38: 691-694.
- Bouksaim M, Lacroix C, Bazin R, Simard RE (1999). ELISA and for immuno-location of in producing and sensitive bacterial strains, J. Appl. Microbiol. 87(4): 500-510.

- Buyong N, Kok J, Luchansky JB (1998). Use of a genetically enhanced, pedio-producing starter culture, *Lactococcus lactis* subsp. *Lactis* MM217, to control *Listeria monocytogenes* in Cheddar cheese. *Appl. Environ. Microbiol.* 64: 4842-4845.
- Capaldo-Kimpal F, Barbour SD (1971). Involvement of recombination genes in growth and viability of *Escherichia coli* K-12. *J. Biotechnol.*, pp. 204-212.
- Cleveland J, Montville TJ, Nies IF, Chikindas MI (2001). Bacteriocins: Safe natural antimicrobials or food preservation. *Int. J. Food Microbiol.* 71: 1-20.
- Davies DG, Chakrabarty AM, Geesey GG (1993). Exopolysaccharide production in biofilms: substratum activation of alginate gene expression by *Pseudomonas aeruginosa*. *Appl. Environ. Microbiol.* 59: 1181-1186.
- Durlu-Ozkaya F, Xanthopoulos V, Tunail N, Litopoulou-Tzanetaki E (2001). Technologically important properties of lactic acid bacteria isolated from Beyaz cheese made from raw ewes milk. *J. Appl. Microbiol.* 91: 861-870.
- Erdogan A, Gurses M (2005). Lactic acid bacteria isolating from blue mouldy Tulum cheese produced with *Penicillium roqueforti*. *Int. J. Food Properties* 8: 405-411.
- Gulsun S, Oguzoglu N, Inan A, Ceran N (2005). The virulence factors and antibiotic sensitivities of *Escherichia coli* isolated from recurrent urinary tract infections. *Saudi Med. J.* 26(11): 1755-1758.
- Holzapfel WH, Geisen R, Schillinger I (1995). Biopreservation by lactic acid bacteria. *Springerlink* 70(2-4): 331-345.
- Hurst A (1981). Nisin. *Adv. Appl. Microbiol.* 27: 85-123.
- Jamuna M, Babusha ST, Jeevaratnam K (2005). Inhibitory efficacy of nisin and bacteriocins from lactobacillus isolates against food spoilage and pathogenic organism because of their production of bacteriocins. *Food Microbiol.* (in press).
- Jamuna M, Jeevaratnam K (2004) Isolation and characterization of lactobacilli from some traditional fermented foods and evaluation of the bacteriocins. *J. Gen. Appl. Microbiol.*, 50: 79-90.
- Jay JM (2000). Fermentation and fermented dairy products, In *Modern Food Microbiology* (6th ed), pp. 113 – 114.
- Kandler O, Weiss N (1986). Genus *Lactobacillus*. In: *Bergey's manual of systematic bacteriology*, Sneath PHA, Mair NS, Sharpe JGH (Eds), Vol. 2, Baltimore: Williams and Wilkins Co., pp. 1209-1234.
- Ogundiwin JO, Oke OL (1983). Factors affecting the processing of 'wara'—A Nigeria white cheese. *Food Chem.* 11: 1-13.
- Pal V, Jamuna M, Jeevaratnam K (2005). Isolation and characterization of Bacteriocin producing lactic acid bacteria from a south Indian special dosa (Appam) batter. *J. Cult Collect.*, 4: 53-60.
- Reed W, Jane G, Long S, Reuber TL, Walker GC (1988). Analysis of the role of exopolysaccharides in *Rhizobium* symbiosis, pp. 51-59. In Keen NT, Kosuge T, Walling LL (ed.), *physiology and biochemistry of plant microbe interactions*. Proceedings of the 11th Annual Symposium in Plant Physiology, University of California. Am. Soc. Plant Physiol, Rockville, Md. pp. 51-59.
- Reinheimer JA, Demkow MR, Condioti MC (1990). Inhibition of coliform bacgeria by lactic cultures. *Austr. J. Dairy Technol.*, pp. 5-9.
- Rodriguez, JM (1996). Review: antimicrobial spectrum, structure, properties and mode of action of Nisin, a bacteriocin produced by *lactococcus lactis*. *Food Sci. Technol.* 2: 61-68.
- Seto A, Saito Y, Matsushige M, Kobayashi H, Sasaki Y, Tonouchi N, Tsuchida T, Yoshinaga F, Ueda K, Beppu T (2006). Effective cellulose production by a coculture of *Gluconacetobacter xylinus* and *Lactobacillus mali*. *Appl. Microbiol. Biotechnol.*, 73(4): 915-921.
- Tarelli GT, Carminati D, Giraffa G (1994) Production of bacteriocins active against *Listeria monocytogenes* and *Listeria innocua* from dairy enterococci. *Food Microbiol* 11: 243-252.
- Updegraff DM (1969) Semimicro Determination of Cellulose in Biological materials. *Anal. Biochem.*, 32: 420-424.
- Zottola EA, Yessi, TL, Ajao, DB, Roberts, RF (1994). Utilization of cheddar cheese containing Nisin as an antimicrobial agents in other Foods. *Int. J. Food Microbiol.*, 24:227-238.