

## Partial Characterization of Bacteriocins Produced by *Lactobacillus reuteri* 2-20B and *Pediococcus acidilactici* 0-11A Isolated from Fura, a Millet-Based Fermented Food in Ghana

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### Abstract

Cell-free supernatants (CFS) produced by 369 LAB strains previously isolated from fura and identified based on sequencing of their 16S rRNA genes were screened for their antagonistic activities against pathogenic bacteria including *Bacillus cereus* PA24, *Escherichia coli* SKN 541, *Enterococcus faecalis* 103907 CIP, *Staphylococcus aureus* ATCC 19095 and *Listeria monocytogenes* Scott A, using the agar well diffusion method. Bacteriocins of *Lactobacillus reuteri* 2-20B and *Pediococcus acidilactici* 0-11A were further evaluated for their stability when subjected to a range of pH conditions, enzymatic and heat treatments. Growth and bacteriocin production rates as well as influence of media composition on bacteriocin production were also evaluated. Cell free supernatants of *Lb. reuteri* and *Pd. acidilactici* strains exhibited the widest inhibitory activities whereas CFS of *Lb. fermentum* exhibited the least inhibitory activity towards the tested pathogens. Bacteriocins of *Lb. reuteri* 2-20B and *Pd. acidilactici* 0-11A retained their antibacterial activities over a wide range of pH. Whereas the antimicrobial activity of *Lb. reuteri* 2-20B was lost after being subjected to temperature of 90°C for 1 h, CFS of *Pd. acidilactici* 0-11A remained stable after autoclaving at 121°C for 15 min. Again, the antimicrobial activities of both *Lb. reuteri* 2-20B and *Pd. acidilactici* 0-11A were lost when the CFSs were subjected to the action of proteolytic enzymes but remained active under the actions of catalase, lipase and  $\alpha$ -amylase. Production of bacteriocins by both *Lb. reuteri* 2-20B and *Pd. acidilactici* 0-11A were growth associated and influenced by media composition.

**Keywords:** bacteriocin, cell free supernatant, lactic acid bacteria, fura, traditional fermentation

### 1. Introduction

Millet (*Pennisetum glaucum*) is a cereal for the production of many common staple foods in West Africa. In Ghana, millet is used for the production of fermented foods such as koko and koko sour water, furo-furo, maasa, and fura. Fura a millet-based spontaneously fermented dumpling is produced in parts of West Africa including Ghana. The microorganisms associated with fura processing have been isolated and identified by both classical and molecular biology-based techniques to include lactic acid bacteria and yeasts (Owusu-Kwarteng et al., 2012).

Lactic acid bacteria (LAB) have a long history of application in fermented foods because of their beneficial influence on nutritional, organoleptic, and safety and preservative characteristics (Hammes & Vogel, 1995; Leroy & De Vuyst, 2004). During fermentation, LAB displays a range of antimicrobial activities due to the production of organic acids and bioactive molecules such as ethanol, formic acid, fatty acids, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and diacetyl. The screening of LAB for antimicrobial activity has shown that, besides acidification, some LAB produce inhibitory substances other than organic acids. Thus, certain strains of LAB produce bacteriocins and bacteriocin-like molecules that display antimicrobial activities (De Vuyst & Vandamme, 1994) which

inhibits the growth of pathogenic and food spoilage microorganisms thereby ensuring safety of the product.

Whereas the involvement of LAB in fura and similar other traditional fermented foods is based on spontaneous fermentations, a deliberate addition of the isolated microorganisms as starters to the food matrix would result in a high degree of control over the fermentation process to yield a more standardized end product. Owusu-Kwarteng et al. (2012) identified the LAB isolated during traditional fura processing in Ghana. However, no study has so far screened the isolated LAB from fura for their ability to produce bacteriocins. The objective of this study was therefore to screen LAB isolated during traditional fura processing for their ability to produce bacteriocins against selected pathogens, and to assess their effectiveness when subjected to some food processing environments. This is geared towards the provision of rational basis for further research in selecting potential LAB strains for use as starter cultures, co-cultures, or bio-protective cultures, to improve the quality and safety of fura and other fermented food products.

## 2. Materials and Methods

### 2.1 Lactic Acid Bacteria

Lactic acid bacteria used in this study were previously isolated during traditional fura processing in Ghana. They were identified by a combination of phenotypic and genotypic methods including conventional morphological characteristics and carbohydrate fermentation profiling, (GTG)<sub>5</sub> – based rep-PCR fingerprinting and 16S rRNA gene sequencing as described elsewhere (Owusu-Kwarteng et al., 2012).

### 2.2 Indicator Strains (Pathogens)

Indicator organisms used in this study and their sources are shown in Table 1.

Table 1. Indicator organisms and their sources/origin

Indicator organisms	Source
<i>Bacillus cereus</i> PA24	Food condiment, Copenhagen University culture collection
<i>Escherichia coli</i> 81 nr.1495 SKN 541	Copenhagen University culture collection
<i>Enterococcus faecalis</i> 103907 CIP	Obtained from Food Research Institute, Ghana
<i>Staphylococcus aureus</i> ATCC 19095	Clinical, American type culture collection
<i>Listeria monocytogenes</i> Scott A, SKN 1014	Copenhagen University culture collection

### 2.3 Preparation of Cell-free Supernatant (CFS)

Each LAB isolate was grown in MRS broth at 30°C for 48 h in a shaking incubator. The culture extracts were obtained by centrifugation at 5,000 g for 10 min. the supernatants were decanted and the pHs adjusted to 6.5 with NaOH (1 mol L<sup>-1</sup>), to eliminate the effect of acidity (Mathieu et al., 1993). Inhibitory activity of hydrogen peroxide was eliminated by the addition of catalase (5 mg ml<sup>-1</sup>, Sigma, C-100 bovine liver) (Daba et al., 1993) and filtered through 0.20 µm pore size syringe filters (Sartorius, Minisart®, Göttingen, Germany).

### 2.4 Screening of LAB for Antagonistic Activity against Pathogenic Bacteria

The agar-well diffusion method was employed in the screening LAB for bacteriocin activities. Indicator lawns were prepared by mixing (inoculating) 20 ml of BHI molten agar media with 100 µl (approximately 10<sup>7</sup> cfu/ml) of an overnight culture of each indicator organism and allowing to solidify in a Petri dish. Wells were cut into the agar with a sterile 6 mm diameter cork borer and sealed with two drops of sterile agar. Fifty microlitres (50 µl) of the filtered cell-free supernatant of test strains were separately placed into the wells. The plates, prepared in duplicate, were kept at 4°C for 24 h (Bonade et al., 2001) to allow pre-diffusion of the CFS into the agar and then incubated at 37°C for 24 h. They were then observed for possible clearing of zones (inhibition zones). The antimicrobial activity was determined by measuring the diameter of the inhibition zones around the well. Results were recorded as no inhibition (-), weak inhibition (+), moderate inhibition (++) and strong inhibition (+++) when the diameter is <1 mm, 1-5 mm, 6-10 mm and >10 mm respectively.

### 2.5 Sensitivity of CFS to pH Changes, Enzyme and Heat Treatment

Sensitivity studies were conducted with CFSs obtained from *Lb. reuteri* 2-20B and *Pd. acidilactici* 0-11A. The effect of pH on the bacteriocins was determined by adjusting the cell-free supernatant to pH 2.0 to 9.0 (at increments of one pH unit) with sterile 1 N HCl or 1 N NaOH. After 2 h of incubation at room temperature, the

samples were readjusted to pH 6.5 with sterile 1 N HCl or 1 N NaOH and the activity determined by the agar well diffusion method. For enzyme treatment, 2 ml of CFS were incubated for 1 h in the presence of 1.0 mg ml<sup>-1</sup> (final concentration) trypsin, pronase E, proteinase K, lipase and  $\alpha$ -amylase and then tested for antimicrobial activity. For heat treatment, the CFS were heated in water bath at 30°C to 100°C (at increments of 10°C) for 1 h, or autoclaved (121°C) for 15 min and tested for their bacteriocin activities using the agar well diffusion method described in section 2.4.

### 2.6 Growth Characteristics and Bacteriocin Production

Growth experiments were carried out in 500 ml ErlenMeyer flask containing 250 ml MRS broth. An overnight pre-culture of *Lb. reuteri* 2-20B and *Pd. acidilactici* 0-11A were used for the inoculations of MRS broths at initial densities of ca 10<sup>3</sup> cfu/ml. At 2 h intervals, samples were removed from the culture and used for viable plate count (cfu/ml), OD (600nm), pH measurements, and antimicrobial activity. The antimicrobial concentration of each sample was estimated using the critical method of dilution. Arbitrary Unit ml<sup>-1</sup> (AU/ml) was calculated as the inverse of the highest two-fold dilution which induced definite inhibition.

### 2.7 Effect of Medium Composition on Bacteriocin Production

The effect of medium composition on bacteriocin production was performed according to Todorov and Dicks (2007) with slight modification. Briefly, *Lb. reuteri* 2-20B and *Pd. acidilactici* 0-11A were separately grown in 10 ml MRS broth for 20 h at 30°C. The cells were harvested by centrifugation (8000xg, 10 min, 4°C), and the pellet re-suspended in 10 ml sterile peptone water. Four ml of the cell suspension was used to inoculate 200 ml of the following media: (a) MRS broth (de Man et al., 1960) without organic nutrients supplemented with tryptone (20.0 g/L), meat extract (20.0 g/L), yeast extract (20.0 g/L), tryptone (12.5 g/L) plus meat extract (7.5 g/L), tryptone (12.5 g/L) plus yeast extract (7.5 g/L), meat extract (10.0 g/L) plus yeast extract (10.0 g/L), or a combination of tryptone (10.0 g/L), meat extract (5.0 g/L) and yeast extract (5.0 g/L), respectively; (b) MRS broth, i.e. with 20.0 g/l D-glucose; (c) MRS broth without D-glucose, supplemented with 20.0 g/L fructose, sucrose, lactose, mannose, and maltose, respectively; (d) MRS broth supplemented with 0.0, 0.1, 0.2, and 0.5 ml/L tween 80 respectively; (e) MRS broth supplemented with 0.0, 1.0, 2.0 and 4.0 g/L glycerol respectively. All cultures were incubated at 30°C (initial pH of 6.5).

### 2.8 Statistical Analysis

Data obtained were subjected to one-way analysis of variance (ANOVA) and means were separated by Tukey's family error rate multiple comparison test (p<0.05) using the MINITAB statistical software package (MINITAB Inc. Release 14 for windows, 2004).

## 3. Results and Discussion

### 3.1 Screening of LAB for Antimicrobial Activities

A total of 369 previously identified strains of LAB (Owusu-Kwarteng et al., 2012) were first screened for their antimicrobial activities. These comprised 176 strains of *Lb. fermentum*, 82 strains of *W. confusa*, 60 strains of *Lb. reuteri* and 51 strains of *Pd. acidilactici* (Table 2). Antagonistic activities of CFSs of the LAB is shown in Table 2. The result demonstrated a diversity of the strains, within and between species, in their ability and the extent to which they inhibit the growth of different pathogenic bacteria. Strains of *Lb. reuteri* and *Pd. acidilactici* displayed the widest inhibitory activities against the indicator pathogens whereas *Lb. fermentum* species showed the least inhibitory activity spectrum against the pathogens. *Staphylococcus aureus* enterotoxin A producer was the most susceptible pathogen with about 57% and 52% strains of *Lb. reuteri* and *Pd. acidilactici* respectively showing inhibition towards *S. aureus* ATCC 19095. Generally, the Gram-positive pathogenic bacteria were more susceptible to the inhibitory actions of the isolated LAB than the Gram-negative indicator organisms.

Several bacteriocin producing LAB have been isolated from various traditional spontaneous fermented foods such as bosa (Todorov & Dicks, 2006), kenkey (Olsen et al., 1995), and ogi and fufu (Sanni et al., 1999; Olukoya et al., 1993). Similarly, strains of *Lb. reuteri* and *Pd. acidilactici* in this study produced bacteriocins which showed inhibition against both the Gram-positive and Gram-negative bacteria (*E. coli* SKN541) although the Gram-positive bacteria were generally more susceptible. Bacteriocins of LAB have been defined as bioactive peptides or proteins that are active against Gram-positive bacteria and usually against species closely related to the producer strain (De Vuyst & Vandamme, 1994). There are however, reports on bacteriocins of LAB with activity against a broad spectrum of Gram-positive and Gram-negative bacteria, including *Klebsiella pneumoniae*, *E. coli*, and *Pseudomonas spp.* (Todorov & Dicks 2005) which are not necessarily closely related to the producer strains.

### 3.2 Effect of Heat, Enzymes and pH Treatments on Activity of Bacteriocins

The effect of different treatments on inhibitory activity of bacteriocins of *Lb. reuteri* 2-20B and *Pd. acidilactici* 0-11A against *S. aureus* ATCC 19095 is shown in Table 3. Inhibitory activity of these bacteriocins reduced significantly ( $p < 0.05$ ) with increasing temperature and was completely inactivated at temperatures above 90°C. The antibacterial activity was lost when the bacteriocins were subjected to the action of proteolytic enzymes such as pronase E, proteinase K, and trypsin but remained active under the action of catalase, lipase and  $\alpha$ -amylase. Both bacteriocins remained active after being subjected to a wide range of pH conditions. The bacteriocin produced by *Lb. reuteri* 2-20B remained active over a wide range of temperatures up to 80°C for 1 h. On the other hand, bacteriocin of *Pd. acidilactici* 0-11A was stable up to 121°C (autoclaving) for 1 h although there was a significant ( $p < 0.05$ ) decrease in activity after autoclaving. It thus suggested that bacteriocin of *Lb. reuteri* 2-20B may not be suitable in foods before they are cooked at temperatures above 80°C and can be considered for usage after cooking if such high temperatures are required. Some other bacteriocins of LAB, especially those of sourdough origins, have displayed temperature stabilities between 90 and 121°C (Van Der Merwe et al., 2004; Mollendorff et al., 2006; Mezaini et al., 2009). On the contrary, the activity of bavaricin MN, another bacteriocin produced by a *Lb. bavaricus* strain isolated from meat, is completely lost after heating at 60°C for 15 min and at 100°C for 10 min (Lewus & Montville, 1992). Bacteriocins of both *Lb. reuteri* 2-20B and *Pd. acidilactici* 0-11A remained active over a wide range of pH conditions although there was reduced activity at more alkaline pH. A similar report has shown that at pH 9.0 and above, there was a significant loss in activity of plantaricin ST31 (Todorov et al., 1999). Notwithstanding, these bacteriocins will remain stable at pH conditions prevailing in most food environments. Constant changes in pH and medium composition during fermentation however led to changes in activity levels of bacteriocins produced by *Lb. mesenteroides* L124, and *Lb. curvatus* L442 (Mataragas et al., 2003). The inactivation of both bacteriocins of *Lb. reuteri* 2-20B and *Pd. acidilactici* 0-11A by proteolytic enzymes while remaining active under the influence of catalase, lipase and  $\alpha$ -amylase was further confirmation of the peptidic or proteinaceous nature of bacteriocins and that the zones of inhibition observed were not due to the action of hydrogen peroxides. Similar observations have been made for other bacteriocins (Todorov & Dicks 2004; Mezaini et al., 2009). Trypsin and pronase E completely inhibited the activity of bavaricin A (Larsen et al., 1993) and plantaricin ST31 (Todorov et al., 1999).

Table 2. Antagonistic activities of predominant lactic acid bacteria isolated from fura

LAB	Indicator organisms				
	<i>S. aureus</i>	<i>B. cereus</i> PA24	<i>E. coli</i> SKN 54	<i>Ent. Faecalis</i> 103907 CIP	<i>L. monocytogenes</i> scott A
<i>Lb. fermentum</i> n= 176	- (82)a + (18) ++ (0) +++ (0)	- (100) + (0) ++ (0) +++ (0)	- (98) + (2) ++ (0) +++ (0)	- (87) + (13) ++ (0) +++ (0)	- (91) + (9) ++ (0) +++ (0)
<i>Lb. reuteri</i> n= 60	- (43) + (28) ++ (10) +++ (8)	- (70) + (30) ++ (0) +++ (0)	- (73) + (27) ++ (0) +++ (0)	- (61) + (26) ++ (8) +++ (5)	- (53) + (36) ++ (6) +++ (5)
<i>W. confusa</i> n= 82	- (71) + (29) ++ (0) +++ (0)	- (91) + (9) ++ (0) +++ (0)	- (95) + (5) ++ (0) +++ (0)	- (74) + (21) ++ (5) +++ (0)	- (82) + (18) ++ (0) +++ (0)
<i>Pd. acidilactici</i> n= 51	- (48) + (31) ++ (9) +++ (12)	- (62) + (35) ++ (3) +++ (0)	- (74) + (22) ++ (4) +++ (0)	- (53) + (32) ++ (7) +++ (8)	- (79) + (13) ++ (0) +++ (8)

Values in parenthesis are percentages of the particular species (n) which showed inhibitions at the levels indicated before them; n: number of strains screened for inhibition; *S*: *Staphylococcus*, *B*: *Bacillus*, *E*: *Escherichia*, *Ent*: *Enterococcus*, *L*: *Listeria*, *Lb*: *Lactobacillus*, *W*: *Weissella* *Pd*: *Pediococcus*, - : no inhibition, + : weak inhibition, ++ : moderate inhibition, and +++ : strong inhibition.

### 3.3 Growth and Bacteriocin Production by *Lb. reuteri* 2-20B and *Pd. acidilactici* 0-11A

Growth kinetics and bacteriocin production by *Lb. reuteri* 2-20B and *Pd. acidilactici* 0-11A were studied in MRS broth at 30°C with initial pH 6.5. Bacteriocin activities were first detected at the exponential growth phase for both *Pd. acidilactici* 0-11A and *Lb. reuteri* 2-20B. Bacteriocin production increased with increasing cell concentration, reaching a maximum of 12800 AU/ml after 12 h and 14 h of growth for *Pd. acidilactici* 0-11A and *Lb. reuteri* 2-20B, respectively (Figure 1). The highest activities were recorded at the stationary growth phases. Bacteriocins of both *Pd. acidilactici* 0-11A and *Lb. reuteri* 2-20B remained active during the stationary growth phase but a reduction of activity was observed after 20 h and 22 h for *Pd. acidilactici* 0-11A and *Lb. reuteri* 2-20B respectively. Other studies have similarly found production of plantaricin ST31 in detectable amounts during the exponential growth phase, while maximum activities (3200 AU/ml) were observed in the stationary phase (Todorov et al., 1999). Again, BLIS C57 activity was detected after 6 h of incubation with a drastic increase at the stationary growth phase. A loss of activity was, however observed after 72 h of growth (Corsetti et al., 1996). A loss of bacteriocins activity after extended period of incubation has been attributed to factors such as proteolytic degradation, protein aggregation, adsorption to cell surfaces, and feedback regulation (Parente & Ricciardi, 1994; Aasen et al., 2000).

Table 3. Effect of different treatments on inhibitory activity of cell-free supernatant of *Lb. reuteri* 2-10B and *Pd. acidilactici* 0-11A against *Staphylococcus aureus* enterotoxin A producer

	Lb. reuteri 2-20B		Pd. acidilactici 0-11A	
<b>Heat treatment (°C)</b>				
30	12.3±1.5a	+++	13.2±1.0a	+++
40	13.0±2.0a	+++	11.7±0.3ab	+++
50	9.6±1.0b	++	11.3±0.8ab	+++
60	7.7±0.6c	++	9.7±0.6b	++
70	4.7±1.2d	+	9.0±0.5b	++
80	4.3±0.8d	+	7.6±0.6c	++
90	0.0±0.0e	-	8.0±1.0c	++
100	0.0±0.0e	-	5.0±1.0d	+
121(Autoclaving)	0.0±0.0e	-	5.7±0.6d	+
Control	13.6±1.2a	+++	12.7±0.3a	+++
<b>Enzymatic treatment</b>				
Catalaze	13.0±1.0e	+++	12.4±0.8e	+++
Lipase	12.7±1.2e	+++	13.2±1.1e	+++
Pronase E	0.0±0.0f	-	0.0±0.0f	-
Proteinase K	0.0±0.0f	-	0.0±0.0f	-
Trypsin	0.0±0.0f	-	0.0±0.0f	-
α-amylase	12.3±2.1e	+++	13.3±1.4e	+++
Control	13.8±1.2e	+++	13.7±0.5e	+++
<b>pH treatment</b>				
3	14.8±1.7h	+++	15.5±1.3h	+++
4	15.3±2.1h	+++	15.1±0.8h	+++
5	12.1±1.2k	+++	15.3±1.1h	+++
6	12.6±0.8k	+++	12.8±1.3k	+++
7	11.5±1.5k	+++	13.2±0.7k	+++
8	12.0±2.0k	+++	12.3±1.2k	+++
9	8.7 ±2.1j	++	4.7±0.8g	+

<sup>1</sup>Values are means of three replicate experiments; ±: standard deviations (SD). Means with different letters as superscripts for each treatment column are significantly different ( $p < 0.05$ ). -: no inhibition, +: weak inhibition, ++: moderate inhibition, +++: strong inhibition, IZ: Inhibition zone.

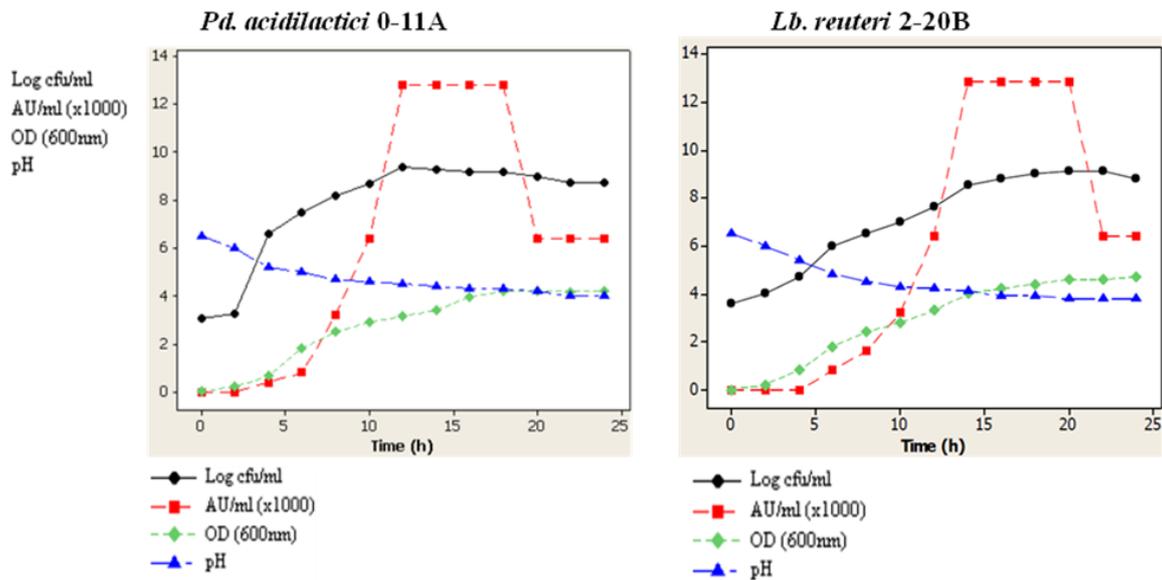


Figure 1. Growth kinetics and bacteriocin production by *Pd. acidilactici* 0-11A and *Lb. reuteri* 2-20B. Growth experiments were conducted at initial pH of 6.5 at 30°C

#### 3.4 Influence of Media Composition on Bacteriocin Production by *Pd. acidilactici* 0-11A and *Lb. reuteri* 2-20B

Growth in basal medium containing tryptone as sole nitrogen source yielded bacteriocin activities of 6400 AU/ml and 12800 AU/ml for *Pd. acidilactici* 0-11A and *Lb. reuteri* 2-20B respectively. For *Pd. acidilactici*, the use of yeast extract, meat extract, or a combination of both reduced bacteriocin activities levels to 800, 3200 and 3200 AU/ml respectively. Similarly, for *Lb. reuteri* 2-20B, the use of yeast extract, meat extract, or a combination of both resulted in reduced bacteriocin activities levels to 1600, 6400 and 3200 AU/ml respectively (Table 4). Tryptone therefore seemed to be the major driver for optimal bacteriocin production by both *Pd. acidilactici* 0-11A and *Lb. reuteri* 2-20B as tryptone alone or in combination with other nitrogen sources yielded the highest bacteriocin activity (12800 AU/ml). Different bacteriocin producing LAB may require specific nitrogen sources for optimal activities. Like reported in this paper, tryptone has been a key nitrogen source required for optimal production of bacteriocins ST151BR (Todorov & Dicks, 2004), ST712BZ (Todorov & Dicks, 2007), and plantaricin 432 (Verellen et al., 1998). Different bacteriocin activity levels were observed for both *Pd. acidilactici* 0-11A and *Lb. reuteri* 2-20B when different carbohydrates were used as the sole carbon sources (Table 4). Glucose as a carbon source stimulated the highest bacteriocin production (12800 AU/ml) for both bacteria. Whereas mannose stimulated the production of bacteriocin by *Pd. acidilactici* 0-11A, bacteriocin production by *Lb. reuteri* 2-20B was reduced to 800AU/ml. Owing to its molecular size, rapid uptake, utilization and cellular energy conversion, glucose has been considered as the usual carbon source for bacterial growth media. However, some bacteria possess abilities owing to their enzymatic activities, to utilize other sugars as carbon sources for growth and for the production of bacteriocins, although bacteriocin activities may vary depending on the carbon source (Todorov & Dicks, 2006). There was a general increase in bacteriocin activities by *Pd. acidilactici* 0-11A and *Lb. reuteri* 2-20B with increasing addition of tween 80 (Table 4). In a tween 80 free medium, bacteriocin activity levels of 400 and 800 AU/ml were observed for *Pd. acidilactici* 0-11A and *Lb. reuteri* 2-20B respectively. However, the addition of 0.1 and 0.2 ml of tween 80 per liter of medium achieved the highest bacteriocin activity (12800 AU/ml) for *Pd. acidilactici* 0-11A and *Lb. reuteri* 2-20B respectively. The influence of tween 80 on bacteriocins activity has been attributed to changes in charges on the cell wall surface of producer strains, thereby preventing adsorption of bacteriocins onto the cells (Mørtvedt-Abilgaard et al., 1995). A reduction in bacteriocin activities were observed with the addition of glycerol (Table 4), an observation which may be due to osmotic stress or reduced water activity. Maximum bacteriocin activities were recorded in the absence of glycerol for both *Pd. acidilactici* 0-11A and *Lb. reuteri* 2-20B.

Table 4. Effect of medium composition on bacteriocin activities of *Pd. acidilactici* 0-11A and *Lb. reuteri* 2-20B

Media composition(g/L)	Quantity	<i>Pd. acidilactici</i> 0-11A	<i>Lb. reuteri</i> 2-20B
		AU/ml	AU/ml
Tryptone (T)	20	6400	12800
Yeast extract (Y)	20	800	1600
Meat extract (M)	20	3200	6400
T + Y	(12,5+7,5)	12800	12800
T + M	(12,5+7,5)	12800	12800
Y + M	(10+10)	3200	3200
T+Y+M	(10+5+5)	12800	12800
Glucose	20	12800	12800
Gluconate	20	800	800
Fructose	20	1600	1600
Mannose	20	12800	800
Maltose	20	1600	1600
Lactose	20	3200	1600
Saccharose	20	1600	800
Tween 80 (ml/L)	0	400	800
	0,1	12800	6400
	0,2	12800	12800
	0,5	12800	12800
Glycerol (g/L)	0	12800	12800
	1	6400	800
	2	3200	800
	4	800	800

#### 4. Conclusions

Some strains of LAB isolated from traditional millet-based fermented food (fura) have demonstrated the ability to inhibit the growth of some food-borne pathogens through the production of bacteriocins and therefore could be exploited for their applications in controlling food-borne pathogenic bacteria. Bacteriocins produced by *Lb. reuteri* 2-20B and *Pd. acidilactici* 0-11A were partially characterized based on their responses to some physicochemical treatments and were influenced by media composition. Since the effectiveness of bacteriocins are dependent on the chemical and physical properties of foods, they must be fully characterized and tested in appropriate food systems before commercial applications.

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#### References

- Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.*, 25, 3389-3402. <http://dx.doi.org/10.1093/nar/25.17.3389>
- Bonade, J. A., Dagnan, A. J., & Garver M. J. (2001). production of helveticin from *Lactobacillus helveticus*. *Lett. Applied Microbiol.*, 33, 153-158. <http://dx.doi.org/10.1046/j.1472-765x.2001.00969.x>
- Caplice, E., & Fitzgerald, G. F. (1999). Food fermentations: role of microorganisms in food production and

- preservation. *Int. J. Food Microbiol.*, *50*, 131-149. [http://dx.doi.org/10.1016/S0168-1605\(99\)00082-3](http://dx.doi.org/10.1016/S0168-1605(99)00082-3)
- Corsetti, A., Gobetti, M., & Smacchi, E. (1996). Antimicrobial activity of sourdough lactic acid bacteria: isolation of a bacteriocin-like inhibitory substance from *Lactobacillus sanfrancisco* C57. *Food Microbiol.*, *13*, 447-456. <http://dx.doi.org/10.1006/fmic.1996.0051>
- Daba, H., Lacroix, C., Huang, J., & Simard, R. E. (1993). Influence of growth conditions on production and activity of Mesenterocin 52 by strains of *Leuconostoc mesenteroides*. *Appl. Microb. Biotechnol.*, *39*, 166-173. <http://dx.doi.org/10.1007/BF00228601>
- De Vuyst, L., & Vandamme, E. J. (1994). Antimicrobial potential of lactic acid bacteria. In F. Leroy, & De L. Vuyst (Eds.), *Bacteriocins of Lactic Acid Bacteria: Microbiology, Genetics and Applications* (pp. 91-142). London, UK: Blackie Academic and Professional. [http://dx.doi.org/10.1007/978-1-4615-2668-1\\_3](http://dx.doi.org/10.1007/978-1-4615-2668-1_3)
- Ennahar, S., Sonomoto, K., & Ishizaki, A. (1999). Class IIa bacteriocins from lactic acid bacteria: antibacterial activity and food preservation. *J. Biosci. Bioeng.*, *87*, 705-716. [http://dx.doi.org/10.1016/S1389-1723\(99\)80142-X](http://dx.doi.org/10.1016/S1389-1723(99)80142-X)
- Gevers, D., Huys, G., & Swings, J. (2001). Applicability of rep-PCR fingerprinting for identification of *Lactobacillus* species. *FEMS Microbiology Letters*, *205*, 31-36. <http://dx.doi.org/10.1111/j.1574-6968.2001.tb10921.x>
- Gänzle, M. G., Hölzel, A., Walter, J., Jung, G., & Hammes, W. P. (2000). Characterization of reutericyclin produced by *Lactobacillus reuteri* LTH2584. *Appl. Environ. Microbiol.*, *66*, 4325-4333. <http://dx.doi.org/10.1128/AEM.66.10.4325-4333.2000>
- Hammes, W. P., & Vogel, R. F. (1995). The genus *Lactobacillus*. In B. J. B. Wood, & W. H. Holzapfel (Eds.), *The Lactic Acid Bacteria* (1st ed) (pp. 19-51). London, UK: Chapman and Hall. [http://dx.doi.org/10.1007/978-1-4615-5817-0\\_3](http://dx.doi.org/10.1007/978-1-4615-5817-0_3)
- Holzapfel, W. H., Geisen, R., & Schillinger, U. (1995). Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. *Int. J. of Food Microbiol.*, *24*, 343-362. [http://dx.doi.org/10.1016/0168-1605\(94\)00036-6](http://dx.doi.org/10.1016/0168-1605(94)00036-6)
- Larsen, A. G., Vogensen, F. K., & Josephsen, J. (1993). Antimicrobial activity of lactic acid bacteria isolated from sour doughs: purification and characterization of bavaricin A, a bacteriocin produced by *Lactobacillus bavaricus* M1401. *J. Appl. Bacteriol.*, *75*, 113-122. <http://dx.doi.org/10.1111/j.1365-2672.1993.tb02755.x>
- Leroy, F., & De Vuyst, L. (2004). Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends Food Sci. Technol.*, *15*, 67-78. <http://dx.doi.org/10.1016/j.tifs.2003.09.004>
- Lewus, C. B., & Montville, T. J. (1992). Further characterization of bacteriocins plantaricin BN, bavaricin MN and pediocin A. *Food Biotechnol.*, *56*, 153-174. <http://dx.doi.org/10.1080/08905439209549829>
- Mataragas, M., Drosinos, E. H., & Metaxopoulos, J. (2003). Antagonistic activity of lactic acid bacteria against *Listeria monocytogenes* in sliced cooked cured pork shoulder stored under vacuum or modified atmosphere at 4±2°C. *Food Microbiol.*, *20*, 259-265. [http://dx.doi.org/10.1016/S0740-0020\(02\)00099-0](http://dx.doi.org/10.1016/S0740-0020(02)00099-0)
- Messens, W., & De Vuyst, L. (2002). Inhibitory substances produced by *Lactobacillus* isolated from sourdoughs – A review. *Int. J. Food Microbiol.*, *72*, 31-34. [http://dx.doi.org/10.1016/S0168-1605\(01\)00611-0](http://dx.doi.org/10.1016/S0168-1605(01)00611-0)
- Mezaini, A., Chihib, N. E., Bouras, A. D., Arroume, N. N., & Hornez, J. P. (2009). Antibacterial activity of some lactic acid bacteria isolated from an Algerian dairy product. *J. Environ. Pub. Health.*, *95*.
- Mollendorff, J. W., Todorov, S. D., & Dicks, L. M. T. (2006). Comparison of bacteriocins produced by lactic-acid bacteria isolated from Boza, a cereal-based fermented beverage from the Balkan Peninsula. *Current Microbiol.*, *53*, 209-216. <http://dx.doi.org/10.1007/s00284-006-0075-9>
- Mørtvedt-Abildgaard, C. I., Nissen-Meyer, J., Jelle, B., Grenov, B., Skaugen, M., & Nes, I. F. (1995). Production and pH dependent bactericidal activity of lactocin S, a lantibiotic from *Lactobacillus sakei* L45. *Appl. Environ. Microbiol.*, *61*, 175-179.
- Nielsen, D. S., Teniola, O. D., Ban-Koffi, L., Owusu, M., Andersson, T. S., & Holzapfel, W. H. (2007). The microbiology of Ghanaian cocoa fermentations analysed using culture-dependent and culture-independent methods. *Int. J. Food Microbiol.*, *114*, 168-186. <http://dx.doi.org/10.1016/j.ijfoodmicro.2006.09.010>
- Olsen, A., Halm, M., & Jakobsen, M. (1995). The antimicrobial activity of lactic acid bacteria from fermented maize (kenkey) and their interactions during fermentation. *J. Appl. Bacteriol.*, *79*, 506-512.

- <http://dx.doi.org/10.1111/j.1365-2672.1995.tb03170.x>
- Olukoya, D. K., Tichazek, P. S., Butsch, A., Vogel, R. F., & Hammes, W. P. (1993). Characterization of the bacteriocins produced by *Lactobacillus pentosus* DK 8 isolated from ogi and *L. plantarum* DK 9 isolated from fufu. *Chem. Mikrobiol. Technol. Lebensm.*, *15*, 65-68.
- Owusu-Kwarteng, J., Akabanda, F., Nielsen, D. S., Tano-Debrah, K., Glover, R. L. K., & Jespersen, L. (2012). Identification of lactic acid bacteria isolated during traditional fura processing in Ghana. *Food Microbiol.*, *32*, 72-78. <http://dx.doi.org/10.1016/j.fm.2012.04.010>
- Owusu-Kwarteng, J., Tano-Debrah, K., Glover, R. L. K., & Akabanda, F. (2010). Process characteristics and microbiology of fura processed in northern Ghana. *Nature and Science*, *8*(8), 41-51.
- Parente, E., & Ricciardi, A. (1994). Influence of pH on the production of enterocin 1146 during batch fermentation. *Lett. Appl. Microbiol.*, *19*(1), 12-15. <http://dx.doi.org/10.1111/j.1472-765X.1994.tb00891.x>
- Riley, M. A., & Wertz, J. E. (2002). Bacteriocins: evolution, ecology, and application. *Ann. Rev. Microbiol.*, *56*, 117-13. <http://dx.doi.org/10.1146/annurev.micro.56.012302.161024>
- Sanni, A. I., Onilude, A. A., Ogunbanwo, S. T., & Smith, S. I. (1999). Antagonistic activity of bacteriocin produced by *Lacobacillus* species from igo, an indigenous fermented food. *J. Basic microbiol.*, *3*, 189-195. [http://dx.doi.org/10.1002/\(SICI\)1521-4028\(199906\)39:3<189::AID-JOBM189>3.0.CO;2-R](http://dx.doi.org/10.1002/(SICI)1521-4028(199906)39:3<189::AID-JOBM189>3.0.CO;2-R)
- Todorov, S. D., & Dicks, L. M. T. (2004). Characterization of mesentericin ST99, a bacteriocin produced by *Leuconostoc mesenteroides* subsp. *dextranicum* ST99 isolated from boza. *Appl. Microbiol. Biotechnol.*, *31*, 323-329.
- Todorov, S. D., & Dicks, L. M. T. (2004). Effect of medium components on bacteriocin production by *Lactobacillus pentosus* ST151BR, a strain isolated from beer produced by the fermentation of maize, barley and soy flour. *World J. Microbiol. Biotechnol.*, *20*, 643-650. <http://dx.doi.org/10.1023/B:WIBI.0000043196.09610.de>
- Todorov, S. D., & Dicks, L. M. T. (2005). Characterization of bacteriocins produced by lactic acid bacteria isolated from spoiled black olives. *J. Basic Microbiol.*, *45*, 312-322. <http://dx.doi.org/10.1002/jobm.200410532>
- Todorov, S. D., & Dicks, L. M. T. (2006). Screening for bacteriocin-producing lactic acid bacteria from boza, a traditional cereal beverage from Bulgaria. Comparison of the bacteriocins. *Process Biochem.*, *41*, 11-19. <http://dx.doi.org/10.1016/j.procbio.2005.01.026>
- Todorov, S. D., Onno, B., Sorokine, O., Chobert, J. M., Ivanova, I., & Dousset, X. (1999). Detection and characterization of a novel antibacterial substance produced by *Lactobacillus plantarum* ST 31 isolated from sourdough. *Int. J. Food Microbiol.*, *48*, 167-177. [http://dx.doi.org/10.1016/S0168-1605\(99\)00048-3](http://dx.doi.org/10.1016/S0168-1605(99)00048-3)
- Van Der Merwe, I. R., Bauer, R., Britz, T. J., & Dicks, L. M. T. (2004). Characterization of thoeniicin 447, a bacteriocin isolated from *Propionibacterium thoenii* strain 447. *Int. J. of Food Microbiol.*, *92*, 153-160. <http://dx.doi.org/10.1016/j.ijfoodmicro.2003.09.004>
- Aasen, I. M., Møretrø, T., Katla, T., Axelsson, L., & Storror, I. (2000). Influence of complex nutrients, temperature and pH on bacteriocin production by *Lactobacillus sakei* CCUG 42687. *Appl. Microbiol. Biotechnol.*, *53*(2), 159-166. <http://dx.doi.org/10.1007/s002530050003>