Gene Identification for Bacteriocin Production by Lactic Acid Bacteria Isolated from Selected Fermented Foods

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Authors' contributions

This work was carried out in collaboration among all authors. Author IHA designed the study, performed the statistical analysis, wrote the protocol, managed the literature searches and wrote the first draft of the manuscript. Authors SSDM and AAO managed the analyses of the study. All authors read and approved the final manuscript.

ABSTRACT

Bacteriocin genes are biosynthetic genes which encodes proteins involved in bacteriocin regulation, self-immunity, transport and modification. This research was aimed at identifying the gene for the synthesis of bacteriocin. Four strains of lactic acid bacteria previously isolated from fermented foods (Nono (N2), Ogi (O3), Dawadawa (D1 and D3) and Wara (W3) were identified using molecular technique and used to produce bacteriocin. The bacteriocin activity was assayed against some test bacteria (Staphylococcus aureus, Escherichia coli, Klebsiella sp and methicillin resistant Staphylococcus aureus) using agar well diffusion method and the bacteriocin genes were identified using BAGEL3. The LAB identified were Lactobacillus fermentum O3, Leuconostoc mesenteroides N2, Weissella cibaria D1 and 2 strains of Lactobacillus plantarum D3 and W3. The entire identified LAB was able to produce bacteriocin. The antimicrobial activity showed varied inhibitory effects of the bacteriocins on the test bacteria. Bacteriocin from isolate O3 showed the highest inhibition zone 16mm on S. aureus. The identified gene for these bacteriocins were plnJK gene for Lactobacillus plantarum str WCFS1 and strain LZ95 (W3 and D3), entA gene for Lactobacillus fermentum str 3872 (O3) and ppnC7 gene for Leuconostoc mesenteroides str SRA3 (N2) with the interaction of other peptides were responsible for bacteriocin production.
Keywords: Bacteriocin; gene identification; lactic acid bacteria; fermented foods; Open Reading Frame (ORF).

1. INTRODUCTION

During fermentation, LAB utilise Carbohydrate substrates available in the fermentation system and produce organic acids, chiefly lactic acid, as a fraction of their metabolites. They produce various substances essential for the development of flavor, aroma, color and texture of fermented foods. Those substances are organic acids, diacetyl, exopolysaccharides, L-alanine, and acetaldehyde. These acids not merely play an important role in the taste and aroma of the product but also lower the product’s pH, which is one of the means to ensure quality and safety. It has been reported that several LAB can produce bacteriocins which are active against closely related species [1].

The conventional factors elaborated by LAB have been identified as “peptides”, generally known as “bacteriocins”. Lactic acid bacteria play a vital role in food fermentation process. Raw foods such as milk, fruits, vegetables or meat are frequently preserved by lactic acid fermentation [2]. In such food products, LAB has the capability to carry out fermentative activities, which may result in dynamic inhibition of pathogenic bacteria. Lactic acid bacteria bacteriocins can work via different mechanisms to exert an antimicrobial effect, but the cell envelope is generally the target.

Bacteriocins are proteins or complex proteins biologically active with antimicrobial action against other bacteria, principally closely related species. Bacteriocins produced by LAB have received considerable attention during recent years for their possible use as biopreservative in foods, with a resultant reduction in the use of chemical preservatives [3]. The bacteria that produce antimicrobial peptides are immune to their own bacteriocins due to the synthesis of specific immunity proteins. According to John and Lennox [4], bacteriocins can be used in three different ways in fermented foods: in situ production by the addition of a bacteriocinogenic lactic culture, as a co-culture and by the direct addition of the bacteriocin.

A typical bacteriocin contains a toxin (bacteriocin) gene, an immunity gene (which confers resistance to the aforementioned toxin) and a lysis gene, which encodes a protein that aids in toxin release from the producing cell [5]. Identification of genes that are functionally similar but have limited or no sequence homology has been a problem, hence the detection of open reading frames ORFs. This is particularly the case for bacteriocins, a very diverse group of antimicrobial peptides produced by bacteria and usually encoded by small, poorly conserved ORFs. ORFs surrounding bacteriocin genes are often biosynthetic genes. This information is used by BAGEL, a web based software to locate putative structural bacteriocin genes [6]. BAGEL scans the bacterial genome for putative bacteriocin open reading frames and also analyses surrounding ORFs to search for possible biosynthetic genes, immunity genes and transporters.

1.1 Bacteriocin Genetics

Bacteriocins can be encoded on chromosomes, plasmids, and other transposable elements. The Gram-positive bacteriocins are more complex genetically, with genes that encode post-translational modification of the toxin. The genetic organization varies between the Gram-positive bacteriocin classes as well – such as the requirement of two peptides for the full activation of Class II bacteriocins. An example is the nisin gene cluster which includes 11 genes (nisABTCIPRKFE) encoding functions such as synthesis of the nisin precursor (nisA), regulation of nisin biosynthesis (nisRK), the processing and translocation of nisin (nisBCTP), and immunity (nisIFEG) [7].

1.2 The Structural Prebacteriocin Gene

The structural gene encodes a prebacteriocin, called a precursor or prepeptide. These prepeptides contain an N-terminal leader sequence and a C-terminal propeptide which is cleaved from the N-terminal leader sequence to form a mature, antimicrobial peptide. All Class II bacteriocins were produced as precursors with an N-terminal extention. Most of the leader peptides differ from typical signal secretion peptides that direct polypeptides into sec-dependent secretion pathways.

The function of leader peptides appears to be the prevention of biological activity of the bacteriocin while still in the producer cell, and to provide a recognition signal for the ABC transporter. Leader peptides may prevent activity of
1.3 The Immunity Gene

The immunity gene encodes a protein that protects the producer organism from its own mature bacteriocin [8]. A close genetic proximity exists between immunity genes and bacteriocin structural and processing genes. In the case of Class II bacteriocins, one gene generally encodes for the immunity protein. The mechanisms involved in immunity are poorly understood, but it has been suggested that interaction between the immunity protein and another protein, perhaps a receptor, located at the cytoplasmic side of the cell membrane of the producer, protects it against the action of the bacteriocin [9,10]. Potential immunity proteins have been identified next to or downstream from, all bacteriocin structural genes studied. Immunity genes not directly associated with the bacteriocin cluster have also been identified. Variation in the presence and expression of these genes may account for the large variation in sensitivity displayed by lactic acid bacteria towards bacteriocins. Immunity proteins range in sizes from 51 to 150 amino acids. While significant homology exists among the structural genes of the Listeria active bacteriocins, this trend does not occur with immunity genes, although some resemblances do occur [8].

1.4 The Transporter Gene

The bacteriocin ABC transporters have a dual function, facilitating both the removal of the leader peptide from its substrate and the transport of the substrate across the cytoplasmic membrane. Bacteriocin ABC-transporters contain three domains on the same polypeptide, consisting of a cytoplasmic N-terminal proteolytic domain, a hydrophobic integral membrane domain, and a cytoplasmic C-terminal ATP-binding domain. Two polypeptides appear to be required for the bacteriocin ABC transporter to be functional.

A unique feature of bacteriocin ABC transporters is that they carry an N-terminal extension of approximately 150 amino acids, the proteolytic domain, which appears to be involved in the processing of the bacteriocins. Two conserved motifs, the cysteine motif (QX4D/ECX2AX3MX4Y/FGx4I/L) and the histidine motif (HY/FY/VVX10I/LXDP) have been identified in the proteolytic domain and appear to be necessary for translocation. The processing site is part of the transporter, which indicates that the processes of cleavage and translocation are integrated, and that the leader peptide serves as a recognition signal for the transmembrane transport process of the bacteriocin [8].

1.5 The Accessory Protein

Several studies have indicated the presence of an additional gene within bacteriocin operons, called the accessory protein (also accessory factor), that is required for the ABC-transporter dependent translocation process. These additional factors have been identified in several Gram-negative systems to be needed when the secreted product is destined for immediate release into the extracellular medium [8]. It is hypothesized that the accessory factor is anchored in the inner membrane and spans the periplasm, probably connecting the inner and outer membranes to facilitate the export of products through both membranes of Gram-negative bacteria. In Gram-positive bacteria, the function of the accessory factor is unclear, since the secreted product only needs to cross one membrane.

The aim of the research is to improve understanding of the mechanisms involved in bacteriocin regulation, processing, translocation and immunity this should facilitate attempts to optimize bacteriocin production and may further open the way to directed in vitro modifications in their antibacterial spectra. Since techniques now available for the site directed mutagenesis of bacteriocin structural genes and with the help of genomics and proteomics, the prospect of constructing new families of designed peptides with improved antimicrobial activity or improved stability and specificity characteristics has become a real possibility.
2. MATERIALS AND METHODS

2.1 Bacterial Strains

The lactic acid bacteria strains used in this study were isolated from fermented foods (nono, ogi, wara and dawadawa) and were identified by using molecular techniques according to the method described by Smith et al. [11].

2.2 Isolation of Lactic Acid Bacteria (LAB)

Solid samples (dawadawa and wara) were homogenized in disinfected grinder (CombinMax600, China) Using sterilized spatula, 25 g of dawadawa, wara, ogi and 25 ml of nono sample were stocked in 225 ml of peptone water to prepare stock solution. 1 ml of the stock was used for serial dilution in peptone water in test tubes to ten folds dilution [12]. To prevent the growth of yeasts, the media was supplemented with 100 mgL\(^{-1}\) of cycloheximide (Ali, 2011). The MRS agar plates were then incubated aerobically and anaerobically using the Gas Pack system at 37°C for 48 h. Discrete Colonies were randomly selected, purify and subsequently stored at 37°C for further identification [13].

2.3 Isolation of Test Bacteria

The test organisms were obtained from the laboratory of Department of Microbiology, Kaduna State University and confirmed using biochemical tests and molecular techniques. Three (3) differential media were used; Mannitol Salt Agar (MSA), Eosin Methylene Blue (EMB) and Salmonella and Shigella agar (SSA). The manufacturer’s instruction was used as a template in preparing the required quantity of the media. The media was allowed to cool to 45°C. 20 mLs of the molten agar was aseptically poured onto sterile petri dishes and allowed to solidify for 30 minutes. Five colonies of the test bacteria were inoculated on the agar plates and four wells of 6.0 mm in diameter were aseptically bored using a sterile cock borer on each agar plate. Aliquots of 50µl of the bacteriocin inoculum were poured in the agar wells in Petri dishes seeded with the bio assay strain (indicator microorganism): Staphylococcus aureus, Escherichia coli, Klebsiella spp and methicillin resistant Staphylococcus aureus. The plates were then incubated at 37°C for 24 hours. Effects of the bacteriocins were assessed by measuring the diameters of zone of inhibition in millimetre (mm) around the wells.

2.4 Bacteriocin Assays

The MRS broth was seeded with 5% inoculums of overnight LAB culture and maintained anaerobically at 30°C for 48 hours. After incubation, the broth was centrifuged at 5000rpm for 10 minutes at 4°C and the cells separated out. The cell free supernatant was adjusted to pH 4.0 using 1N NaOH and was used as crude bacteriocin [15].

2.5 Partial Purification of the Bacteriocins Produced by LAB

The bacteriocin produced were filtered and precipitated by using 80% ammonium sulphate and the system was held overnight at 4°C. The precipitates were recovered by centrifugation at 15,000 rpm for 30 minutes at 4°C and the pellets were dissolved in 5 ml of 0.05 M potassium phosphate buffer and were designated as crude bacteriocin [16].

2.6 Antagonistic Effect of the Bacteriocins on Selected Bacteria Isolates

To determine the bacteriocin activity, well assay procedure of Udhayashree et al. [2], 20 mLs of Muller-Hinton agar was poured into sterilized Petri dishes and was allowed to solidify for 30 minutes. Five colonies of the test bacteria were inoculated on the agar plates and four wells of 6.0 mm in diameter were aseptically bored using a sterile cock borer on each agar plate. Aliquots of 50µl of the bacteriocin inoculum were poured in the agar wells in Petri dishes seeded with the bio assay strain (indicator microorganism): Staphylococcus aureus, Escherichia coli, Klebsiella spp and methicillin resistant Staphylococcus aureus. The plates were then incubated at 37°C for 24 hours. Effects of the bacteriocins were assessed by measuring the diameters of zone of inhibition in millimetre (mm) around the wells.

2.7 Genomic Extraction

The bacterial DNA was extracted and identified using the method by Smith et al. [11].

2.8 Identification of the Bacteriocin Gene

The bacterial genome sequence was used as input to detect the gene responsible for bacteriocin production using bagel software (a bacteriocin genome mining tool). BAGEL uses DNA nucleotide sequences in FASTA format as input; multiple sequence entries per file allowed. These DNA sequences are analyzed in parallel using two different approaches, one based on finding genes commonly found in the context of bacteriocin or RiPP genes, the other based on finding the gene itself. The degree of novelty in the bacteriocins identified was determined by BAGEL3 and BLASTP searches for each putative bacteriocin peptide against those identified in the BAGEL screen. The genomic
sequences were identified using the NCBI database and the reference sequences generated.

3. RESULTS

3.1 Identification of Lactic Acid Bacteria

The lactic acid bacteria were identified as Weissella cibaria CBA3612, Lactobacillus planterum LZ95 which were isolated from dawadawa, Leuconostoc mesenteroides SRA3 isolated from nono, Lactobacillus fermentum 3872 isolated from ogi and Lactobacillus plantarum WCFS1 from wara.

3.2 Bacteriocin Assay

All the lactic acid bacteria identified produced bacteriocin. The bacteriocin produced had varying inhibitory activity against the indicator bacteria, as shown in Table 1. Lactobacillus plantarum strain WCFS1 (isolate W3) showed zone of inhibition on the entire test organism (S. aureus, E. coli, methicillin resistant S. aureus and K. pneumonia) and bacteriocins from Lactobacillus plantarum strain LZ95 (D3) showed the highest inhibition zone on E. coli (10 mm), while Lactobacillus fermentum (O3) showed the highest inhibition zone on S. aureus (16 mm).

3.3 Identified Gene for Bacteriocin Production

The Lactobacillus plantarum WCFS1 genome contains a six gene, 5941 bp Plantaricin cluster (Table 2). Containing genes predicted to encode a plantaricin structural protein. Immediately upstream are bacteriocin-related genes, including a putative LanM-like lantibiotic modification protein, a putative LanT-like lantibiotic transport protein, ATP-binding and permease protein and some plantaricin immunity protein as shown in (Plate 2).

![Plate 1. Amplified 16S rRNA gene of the PCR products](image)

**Plate 1. Amplified 16S rRNA gene of the PCR products**

*Key: M: 100 bp DNA ladder; -VE: negative control; lane D (dawadawa), lane N (nunu), lane O (ogi), lane W (wara)*

| Sources | pH | Bacteriocin production | S. aureus | E. coli | MRSA | K. pneumonia |
|---------|----|------------------------|-----------|---------|------|-------------|
| N2      | 2.4 | +                      | -         | -       | 14   | 8           |
| N4      | 2.4 | -                      | -         | -       | -    | -           |
| D1      | 2.2 | +                      | -         | 10      | -    | 10          |
| D2      | 2.4 | +                      | 8         | 7       | 8    | -           |
| D3      | 2.5 | +                      | 7         | 16      | -    | 8           |
| D4      | 1.7 | -                      | -         | -       | -    | -           |
| O3      | 3.0 | +                      | 10        | 7       | 8    | -           |
| W1      | 2.2 | -                      | -         | -       | -    | -           |
| W2      | 2.0 | -                      | -         | -       | -    | -           |
| W3      | 1.9 | +                      | 7         | 7       | 14   | 7           |
| W4      | 2.0 | -                      | -         | -       | -    | -           |

D1= Weissella cibaria, D3= Lactobacillus plantarum, O3= Lactobacillus fermentum, W3= Lactobacillus plantarum, N2= Leuconostoc mesenteroides, positive (+), negative (-) MRSA= (Methicillin Resistant Staphylococcus aureus)
Plate 2. Line diagram of predicted lantibiotic gene clusters (Isolate W3; *Lactobacillus plantarum* WCFS1). This diagram was drawn approximately to scale using data from genome sequences. Genes encoding products predicted to resemble known lantibiotic-associated proteins are in gray. Putative structural peptides are in green.

Plate 3. Line diagram of predicted lantibiotic gene clusters (Isolate D3; *Lactobacillus plantarum* LZ95). This diagram was drawn approximately to scale using data from genome sequences. Genes encoding products predicted to resemble known lantibiotic-associated proteins are in gray. Putative structural peptides are in green.
Plate 4. Line diagram of predicted lantibiotic gene clusters (Isolate O3; \textit{Lactobacillus fermentum} 3872). This diagram was drawn approximately to scale using data from genome sequences. Genes encoding products predicted to resemble known lantibiotic- associated proteins are in gray. Putative structural peptides are in green.

Table 2. Bioinformatics analysis of the predicted protein encoded by \textit{Lactobacillus plantarum} WCFS1 (W3) genome

| Name               | ORF   | Gene start | Gene end | Strand | Function                          | Annotation                                                                 | Gene ID | Protein | Gene   | Molecular weight |
|--------------------|-------|------------|----------|--------|-----------------------------------|---------------------------------------------------------------------------|---------|---------|---------|------------------|
| 173.2;Plantaricin_K | orf00024 | 358049     | 358222   | -      | BacteriocinIIc; 173.2; Plantaricin_K | Bacteriocin class II with double-glycine leader peptide; \textit{Evalue}=2e-37 | 106418  | 55      | plnJK   | 6105             |
| 172.2;Plantaricin_J | orf00025 | 358253     | 358420   | -      | Plantaricin_J                     | \textit{Evalue}=6e-37; \textit{match}=100.00%                            | 106418  | 55      | plnJ    | 5941             |
| 174.2;Plantaricin_N | orf00030 | 359610     | 359777   | +      | Bacteriocin_IIc; 174.2; Plantaricin_N | Bacteriocin class II with double-glycine leader peptide; \textit{Evalue}=3e-35 | 106418  | 55      | plnN    | 5828             |
| 167.2;Plantaricin_A | orf00035 | 362749     | 362895   | -      | Antimicrobial17; 167.2;Plantaricin_A | \textit{Evalue}=3e-29                                                  | 106417  | 48      | plnA    | 5326             |
| 171.2;Plantaricin_F | orf00047 | 367550     | 367355   | -      | ggmotif; Lactococcin; Bacteriocin_IIc; 171.2 | Lactococcin-like family with double-glycine leader peptide; \textit{Evalue}=6e-35 | 106128  | 52      | plnF    | 5601             |
| Name               | ORF         | Gene start | Gene end | Strand | Function                                      | Annotation                                                                 | Gene ID | Protein | Gene | Molecular weight |
|--------------------|-------------|------------|----------|--------|-----------------------------------------------|---------------------------------------------------------------------------|---------|---------|------|------------------|
| Plantaricin F     | orf00049    | 367380     | 367550   | -      | 170.2;Plantarici n_E                         | Evalue=7e-37                                                              | 106417  | 56     | plnE | 6060             |
| LanT               | orf00051    | 367817     | 369967   | +      | Bacteriocin ABC-transporter, ATP-binding and permease protein PlnG | Species=Lactobacillus plantarum | | | |
|                    |             |            |          |        |                                               | match=100. | | | |
|                    |             |            |          |        |                                               | Evalue=0.0 | | | |

Table 3. Bioinformatics analysis of the predicted protein encoded by *Lactobacillus plantarum* LZ95 (D3) Genome D3; *Lactobacillus plantarum* LZ95 29495 bp DNA linear

| Name               | ORF         | Gene start | Gene end | Strand | Function                                      | Gene              | Molecular weight | Protein id |
|--------------------|-------------|------------|----------|--------|-----------------------------------------------|-------------------|------------------|------------|
| 185.2;PlnK_(putative) | orf00022    | 367378     | 367551   | -      | 185.2;PlnK_(putative)                        | plnK              | 6105             | ALV13576.1 |
| 172.2;Plantaricin_J | orf00023    | 367180     | 367347   | -      | 172.2;Plantaricin_J                           | plnJ              | 5941             | ALV13577.1 |
| 174.2;Plantaricin_N | orf00030    | 365823     | 365990   | +      | Bacteriocin_Ilc 174.2;Plantaricin_N           | 5828              | ALV13579.1 |
| 167.2;Plantaricin_A | orf00034    | 362705     | 362860   | +      | Antimicrobial!Bacteriocin_Ilc; 167.2;Plantaricin_A | 5326              | ALV13583.1 |
| 171.2;Plantaricin_F | orf00046    | 358245     | 358403   | -      | ggmotif; Lactococcin; Bacteriocin_Ilc; 171.2;Plantaricin_F | pInF              | 5601             | ALV13588.1 |
| 170.2;Plantaricin_E | orf00048    | 358050     | 358220   | -      | 170.2;Plantaricin_E                           | pInE              | 6060             | ALV13589.1 |
| LanT               | orf00050    | 355633     | 357783   | +      | Bacteriocin ABC-transporter, ATP-binding and permease protein PlnG | 5828              | ALV13590.1 |

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Table 4. Bioinformatics analysis of the predicted protein encoded by *Lactobacillus fermentum* 3872 genome

| Name          | ORF    | Gene start | Gene end | Strand | Function   | Annotation       | Gene | Protein id   | Molecular weight |
|---------------|--------|------------|----------|--------|------------|------------------|------|--------------|------------------|
| O3; *Lactobacillus fermentum* gene 20588 bp DNA linear | orf00018 | 835633     | 836847   | +      | Enterolysin_A | Evalue=4e-37   | enlA | AKM52235.2   | 42499            |

Table 5. Bioinformatics analysis of the predicted protein encoded by *Leuconostoc mesenteroides* Genome N2; *Leuconostoc mesenteroides* str C7 linear 2065bp

| Name          | ORF    | Gene ID   | Gene start | Gene end | Protein ID | Protein | Molecular wt | Function |
|---------------|--------|-----------|------------|----------|------------|---------|--------------|----------|
| Orf6          | AAL77869 | 1         | 186        |          | 62         | Unknown |              | Unknown  |
| Orf2          | AAL77868 | 94        | 324        |          | 76         | Uncharacterized |         |              |
| orf3          | AAL77873 | 669       | 824        |          | 51         | Unknown |              | Unknown  |
| Leucocin_K    | Orf6   | AAL77872  | 1083       | 1241     | PpnC7      | 52      |              | Synthesis |
| Orf7          | AAL77875 | 1238      | 1405       |          | 55         | Unknown |              | Unknown  |
| Orf1          | AAL77871 | 1265      | 1420       |          | 51         | Unknown |              | Unknown  |
| Orf4          | AAL77874 | 1518      | 1625       |          | 35         | Unknown |              | Unknown  |
| Orf5          | AAL77870 | 1741      | 1929       |          | 62         | Unknown |              | Unknown  |
**Lactobacillus plantarum** LZ95 genome also contains six precursor bacteriocin gene, 5941 bp plantaricin cluster (Table 3). The area of interest contained genes predicted to encode plantaricin similar to stain WCFS1 genome as shown in (Plate 3).

**Lactobacillus fermentum** 3872 genome contained a putative enterolysin structural protein as shown in (Table 4), enterolysin A is a high molecular weight bacteriocin (42499) together with other uncharacterized protein, membrane peptidase and transposase for insertion element (Plate 4).

**Leuconostoc mesenteroides** C7 genome contained a putative leucocin structural gene (Table 5). This genome contains an 8 gene 2065 bp cluster. Gene PpnC7 is predicted to encode the structural protein Leucocin K.

### 4. DISCUSSION

The production of the bacteriocins were achieved in mixed population, *E. coli* and *Klebsiella pneumonia* were added to the broth to achieve a competitive environment. Similar observations by Ogunbonwa et al. [17] also noted that bacteriocins are often produced under stress conditions, such as nutrient limitation and overpopulation. The prevalence of bacteriocin positive strains in the food samples analysed as shown are much higher than those reported by other findings. Sezer and Güven [18] screen 12,700 LAB isolates from milk and meat products and found only 35 exhibited bacteriocin production. Also, Salasiah et al. [19] screened 3000 colonies of LAB isolated from traditional fermented foods and only one colony was found to produce inhibitory zone. The high incidence of bacteriocin producing LAB in these foods suggest that they may represent an abundant source of potentially useful bacteria.

Bacteriocins from the LAB had varying inhibitory activity against four indicator bacteria (methicillin resistant *S. aureus, E. coli, S. aureus, K. pneumonia*), as shown. **Leuconostoc mesenteroides** (N2) showed the highest antagonistic effect against methicillin resistant *S. aureus but other bacteriocins from Lactobacillus plantarum WCFS1, Lactobacillus fermentum, Weissella cibaria and Lactobacillus plantarum LZ95 were effective against *E. coli, S. aureus, K. pneumonia*. Bacteriocins from isolates D3 and W3 (Lactobacillus plantarum) showed high activity on *E. coli*. A similar observation was made by Hartnett et al. [20] in raw and malted cereals.

Six genes (Plantaricin K, N, A, E, F and J) for precursor bacteriocin and a transporter gene for bacteriocin ABC transporter was detected in both the Lactobacillus plantarum sequences Plasmid sequence analysis of Lactobacillus plantarum LZ95 (D3) revealed six open reading frames (ORFs) containing one replication protein (ORF00023), three hypothetical proteins (ORF00022, ORF00034 and ORF00048 ORF5), one conserved hypothetical protein (ORF00046) and one mobilization protein (ORF00050). The predicted protein from ORF00023 (putative replication protein) consisted of fifty five (55) amino acid residues, with a weight of 5,941 Da. Also, it is thermostable, stable at a pH of 5.0 to 8 and has a broad inhibition spectrum against Gram positive and Gram negative bacteria.

The gene for *Lactobacillus plantarum* WCFS1 and *Lactobacillus plantarum* LZ95 exhibits antimicrobial activity that was subsequently found by six peptides with fifty five (55) amino acid residue and molecular weight of (5941 DA). The gene for this bacteriocin was identified as plnJK and the bacteriocin produced was plantaricin. Wang et al. [21] isolated *Lactobacillus plantarum* with similar gene cluster but differ in their molecular weight and hydrophobicity. Gene plnK of these isolates (W3 and D3) were 100% identical to the *Lactobacillus plantarum* under the accession number WP-003641972, isolated by Nissen-Meyer et al. [22]. *Lactobacillus fermentum* produced enterolysin bacteriocin and the gene was identified as enlA gene with 404 amino acid residue and 42499 DA molecular weight. Similar report by Nilsen et al. [23] identified Enterolysin A isolated from *Enterococcus faecalis* which was 56% positive, 41% identical and had 38% similar proteins. Another report by Altermann et al. [24] identified gene LBA1207 with similar properties. Isolate N2 produced leucocin K bacteriocin and gene ppnC7 with 52 amino acid was identified.

Sequencing of the entire locus of Lactobacillus plantarum WCFS1 showed it to comprise 62 ORF, while Lactobacillus plantarum LZ95 was 65 ORF, then Leuconostoc mesenteroides had 7 ORF and 37 ORF for Lactobacillus fermentum. Sequence analysis and BLAST homologies indicated these are likely to be involved in lantibiotic biosynthesis, regulation, immunity and N-terminal peptide. Weissella cibaria (D1) gene of interest was not identified by the database as
it does not have strong homology (partially sequenced) to previously identified peptides. It was also reported that the GenBank database on Weissella genes is too short to predict or identify their specific functions. From that research, five of six ORFs in pKW2124 were not predicted properly, probably due to low GenBank information on Weissella genes [25].

Bacteriocin genes identified belong to class II (a) and (b) bacteriocins due to the presence of double glysine motifs detected by BAGEL. An interesting structural feature of all Class IIb bacteriocins is the presence of GxxxG motifs (where x represents any residue) and GxxxG type motifs [conformed by A (Ax Ax A) or S (SxxxS)] that facilitate helix-helix interactions and promote the oligomerization of transmembrane helical peptides or membrane protein domains as reported by several authors [26,27]. Although E-760 shares this Class IIb uniqueness, it lacks the second characteristic peptide of this category; since in order to achieve an optimal antibacterial activity, the presence of both peptides is required in approximately equal amounts.

5. CONCLUSION

From the result, it was observed that for the full activation of bacteriocins in Gram positive lactic acid bacteria the interaction of other genes; biosynthesis, regulation, immunity and transport may be required. As also stated by Bakkal et al. [5] who reported that Class II bacteriocins require two peptides for their full activation.

ACKNOWLEDGEMENTS

The authors appreciate Kaduna State University management for provision of laboratories that were appropriate to achieve this work. The efforts of Mr Ahmed Yahaya to provide funds to ensure the manuscript for this paper was prepared and processed is also appreciated.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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