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Assessing the Biopreservative Potential of Bacteriocin against Some Selected Food Pathogens

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Abstract:*Bacteriocins are antimicrobial peptides or proteins ribosomally synthesized by some lactic acid bacteria (LAB). This research study was carried out to assess the potential of bacteriocin from Lactobacillus acidophilus (MS1), Bacillus cereus (MS2) and Staphylococcus epidermidis(MS3) against selected food borne pathogens. The bacteriocin producing bacteria was isolated from animal samples pre-enriched in broth culture of Nutrient agar for 48hrs at 30^{o}C. the biochemical and morphological properties were used to characterize and identify the isolates as Lactobacillus acidophilus, Bacillus cereus and Staphylococcus epidermidis. Crude extract of bacteriocin was obtained by centrifugation of the broth culture (4000r/min) for 20mins. Extract was further purified by precipitation with ammonium sulfate and sterilized by filtration using Whatmann filter paper no. 12. The sterile and pure bacteriocin was labeled as MS1, MS2 and MS3. Bacteriocin was quantified using spectrophotometer at 450nm to achieve a standard curve of R^2=0.9761.Bacteriocinactivity shows that MS1 was higher with 35Au/ml than MS2 with 27Au/ml and MS3 with 23Au/ml. Antibacterial activity showed that (MS1) was effective against Salmonella typhi, Listeria monocytogenesand Brochothrixthermosphacta , (MS2) was effective against Brochothrixthermosphacta and MS3 was effective against Listeria monocytogenes with significant activity starting from 2mm and above. This research revealed that, Lactobacillus acidophilus (MS1), Bacillus cereus MS2 and Staphylococcus epidermidis (MS3) are good bacteriocin producing bacteria and are effective as biopreservative against predominant food pathogens and hence, provide alternative solution to problems associated with chemical preservatives. Further studies are recommended to assess the proper dose and administration route of these biopreservatives for commercial purpose.*

Key word: *Bacteriocin, Biopreservative, Food pathogens, LAB, Bacillus spp*

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I. Introduction

One of the concerns in food industry is the contamination by pathogens, which are frequent cause of food borne diseases. Over the past decade, recurrent outbreaks of diarrhea, coupled with the natural resistance of the causative agents, contributes to its status as hazard (Scallanet al., 2011). The problem of selection of resistant bacteria to antibiotics and the increasing demand for safe foods, with less chemical additives, has increased the interest in replacing these compounds by natural products, which do not injure the host or the environment. Biotechnology in the food-processing sector targets the selection, production and improvement of useful microorganisms and their products, as well as their technical application in food quality (Kapil, 2005).

Foodborne illness caused by consumption of food contaminated with pathogens or spoilage bacteria is of great concern in public health. Many known pathogens such as Bacillus cereus, Campylobacter spp., Listeria monocytogenes, Salmonella sp., Staphylococcusaureus, Escherichia coli, etc. are responsible for numerous illnesses and death (Scallanet al., 2011).

Bacteriocins are antimicrobial peptides or proteins ribosomally synthesized by some Lactic Acid Bacteria (LAB) (Cleveland et al., 2001). Bacteriocins are non-toxic to eukaryotic cells and are generally recognized as safe substances. Several classes of bacteriocins have been described, including lantibiotic (class I), small heat-stable non-lanthionine peptides (class II), large heat-labile bacteriocins (class III) and complex proteins that require the participation of carbohydrate or lipid moieties to express activity (class IV) (Cleveland et al., 2001).

The use of nisin as a food biopreservative is limited because of its lesser effect against Gram negative bacteria (Arqueset al., 2004). Nisin is produced by some strains of Lactococcuslactis (Thomas and Delves-Broughton, 2005). Enterocins can prevent the growth of many foodborne and spoilage bacteria such as Staphylococcus aureus, Listeria monocytogens Escherichia coli, Pseudomonas spBacciluspp and Clostridium spp. (Franz et al., 2007). Because enterocins are heat stable and active over a wide pH range, they can be used to

enhance the shelf life of different food products. Among different species of Enterococcus that are able to produce bacteriocins for food preservation, E. faecium and E. faecalis are predominant (Javedet al., 2011). Recent years, antimicrobial resistance of many foodborne pathogens to current antibiotics or antimicrobial agents is a great concern of public health (Walsh and Fanning, 2008).

Since genes encoding antimicrobial resistance are frequently associated to mobile genetic elements such as plasmids, transposons, and integrons; spreading of antibiotic resistance genes among bacteria, including bacteria causing infection in animal or humans, can be occurred (Sunde and Nordstrom, 2006). The resistance of bacteria to bacteriocins can also be occurred spontaneously (Bouttefroy and Milliere, 2000). It is expected that when microorganisms are treated by multiple antimicrobial agents, the capacity of their survival could be decreased due to synergic effects of combined antimicrobial agents. Previous study demonstrated that nisin, pediocin, and two enterocins had antibacterial effects against several food borne and spoilage bacteria (Turgiset al., 2012).

Bacteriocins are proteinaceous toxins produced by bacteria and some archaea members to inhibit the growth of similar or closely related bacterial strain(s). The inhibitory spectrum of bacteriocins can be narrow and confined to closely related species, or it can be relatively broad, inhibiting a range of target organisms (Mantovaniet al., 2011). Novel alternative strategies to reduce or eliminate animal pathogens have also been tested by different research groups. The alternatives include bacteriocins, probiotic microorganisms and bacteriophages (Bedford, 2000; Joerger 2003). We assess the potential of bacteriocin from Lactobacillus acidophilus (MS1), Bacillus cereus (MS2) and Staphylococcus epidermidis(MS3) against selected food borne pathogens.

II. Materials And Methods

Sample Processing

Meat sample were collected and aseptically cut into smaller pieces using surgical blade. One gram of the sample was taken for the study and was serially diluted in 9mls of sterile physiological saline.

Bacteriological analysis of meat sample

To isolate the bacteria, 1ml of meat homogenate was mixed in sterile physiological saline and serially diluted up to 10^{-5}. One mililitre of the 10^{-3} dilution was then inoculated into plates of Nutrient Agar (NA). The culture was then incubated at 37^{o}C for 24hours.The population densities of the meat microorganisms were determined by standard plate count which was carried out in nutrient agar. Plates incubated were counted after 24hours. The isolates were gram stain to visualize the cell arrangement and shape of the isolates as described by (Fawole and Oso, 2004).

Identification of the isolates

Morphological colonial characterizations of the isolates were observed from the parent plates for preliminary identification. The followings biochemical tests were carryout in order to identify the isolates capable of producing bacteriocins according to standard procedures as Catalase test, Indole test, Starch Hydrolysis,Urease test, Citrate test (Cheesbrough, 2006).Coagulase test, Methyl red test Voges – Proskaeur test (Olutiolaet al., 2000) and Sugar Fermentation (fawole and Oso, 2004). After characterization the isolate reactions were compared to those in Bergy Manual of Determinative Bacteriology 2nd edition for identification.

Purification and Quantification of Bacteriocin

Bacteriocin producing bacteria were grown in Nutrient broth, for 48hours at 30°C. Crude bacteriocin preparation was obtained by centrifugation of the culture (4,000r/min) for 20mins and was sterilized using Whattman filter no. 12. The extracted bacteriocin were purified by adding 5 drops of ammonium sulphate and quantified using spectrophometry at the wavelength of 450nm and compared to the standard curve chart (R^2 = 0.9716) to determine the concentration of bacteriocin (Coventry, 1996).

Inhibitory Activity

The antibacterial activity of bacteriocin was tested against the test organism following the method described by (Todorov and Dicks, 2004). Isolates were inoculated into MRS broth and incubated at 30^{o}C, without aeration until mid-logarithmic phase of growth. Aliquots of 10ul cell free culture supernatant was spotted on the surface of the agar plates seeded with actively growing cells of the test organism. Plates were incubated at the optimal growth temperature of the test organisms.

III. Results And Discussion

A total of six species of bacteria were isolated and identified as Staphylococcus epidermidis (isolate 1), Bacillusfirmus(isolate 2), Lactobacillus acidophilus (isolate 3), Bacillus cereus (isolate 4), Listeria monocytogenes(Isolate 5) and Staphylococcus chromogenes(isolate 6).The results for the species isolated from meat sample include Staphylococcus epidermidis, Bacillus firmus, Lactobacillus acidophilus, Staphylococcus chromogenesand Listeria spp. This is similar to the findings of Wan et al. (1995) that isolated

Carnobacteriumsp, Enterococcus faecumLactococcuscurvatus, Staphylococcus epidermidis, and Staphylococcusaureus from meat sample.

The bacteria load of bacteriocin producing isolates from fresh meat sample showed that Lactobacillus acidophilus has more bacterial load than Bacillus cereus at 1.8 x 10^5cfu/g and 1.6 x 10^5cfu/g respectively (Fig:1).Bacillus cereus was active against Bacillus subtilis which corresponds to the findings of Jadamuset al., (2002) who observed the activity of Bacillus cereus against Bacillus subtilis. In comparison with some bacteriocins produced by lactic acid bacteria, B. cereus bacteriocins are not heat stable. For example, Lactobacillus acidophilus LF221 could at least partially preserve their activity even after treatment at 100^{o}C for 30mins (Lee et al., 2001). Although B.cereus is considered as potential pathogen microorganism, the properly identified and tested non – toxic strains are most frequently used as animal probiotics (Jadamuset al., 2002).

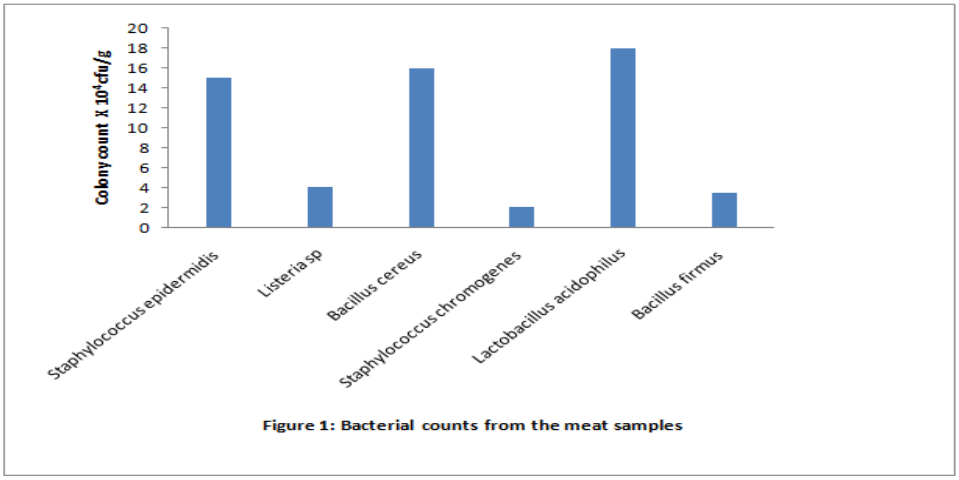

Figure 1: Bacterial counts from the meat samples

Table 1: Bacterial counts from the meat samples

The bacterial load of bacteriocin producing isolates from meat sample shows that Lactobacillus acidophilus has more bacterial load than Staphylococcus epidermidis and Bacillus cereus. This is similar to the findings of Osmanagaogluet al. (1999) which showed that Pediococcusacidilactici isolated from meat fermented sausage is effective against many bacteria associated with food spoilage and food related hazards.

According to Kellneret al. (1988) Staphylococcus epidermidiswhich is the bacteria that produces the bacteriocinepidermin is among the members of the family of lantibiotics that bind lipid II, a cell wall precursor lipid component of target bacteria and disrupt the cell wall production. The type Alantibiotics are long flexible molecules e.gNisin, bisin, subtilin, epidermin and gallidermin. According to Van Kraaijet al. (1999), Lantibiotics are produced by gram positive bacteria and show a strong antimicrobial action toward a wide range of other gram positive candidates for use in food preservation and the pharmaceutical industry.

Table 1: Morphological and biochemical characters of isolates from meat sample

Tests	**Isolate 1**	**Isolate 2**	**Isolate 3**	**Isolate 4**	**Isolate 5**	**Isolate 6**
Gram Reaction	Gram Positive Cocci in Pair	Gram Positive Long rod in cluster	Gram Positive Long rod in clusters	Gram Positive Long rod in chains	Gram Positive Long rod in clusters	Gram Positive Long rod in clusters
Catalase	+	+	-	-	+	+
Coagulase	-	N/A	N/A	N/A	**N/A**	-
Methly red	+	+	-	-	+	-
V.P	-	-	+	+	-	+
Citrate	-	-	+	+	+	-
Indole	+	-	-	-	+	+
Motility	-	-	-	+	+	+
Glucose	+	-	+	+	-	-
Lac	+	+	+	+	+	+
Suc	+	-	+	+	-	-
Gas	-	-	-	+	-	-
H_2S	-	-	+	-	-	-
Starch	N/A	+	-	+	-	**N/A**
Urease	+	-	-	+	-	-

Key: NA = Not available, (+) positive, (-) = negative

The antibacterial activity, showing zones of inhibition of the bacteria isolated from fresh meat. Lactobacillus acidophilus was effected against food pathogen Salmonella typhi, Escherichia coli, Listeria monocytogenes, and Bacillus cereus was effective against the food pathogen Brochotrixtheromosphactawhereas Staphylococcus epidermidiswas effective against the food pathogen Listeria monocytogenes with zones of inhibition greater than 2mm in diameter indicating a positive (Table 3).Lactobacillus acidophilus was active against Salmonella typhi, Escherichia coli, and Listeria monocytogenes. This is in agreement with the finding of (Todorov and Dicks, 2004) who observed the inhibitory activity of lactobacillus acidophilus on salmonella typhi, Escherichia coli and Listeria monocytogenes. Staphylococcus epidermidis was active against Listeria monocytogens. This concurred with the finding of Moreno et al., (2000) who observed that Staphylococcus epidermidis was active against Listeria monocytogens.

Table 2 Antibacterial/Inhibitory activity of Bacteriocin producers isolated from meat

Bacteriocin Code	Bacteriocin Producers	Test Organism	Result
MS1	Lactobacillus acidophilus	Salmonella typhi	4mm
		Listeria monocytogenes	6mm
		Escherichia coli	2.5mm
MS2	Bacillus cereus	Brochothrixthermosphacta	3.5mm
MS3	Staphylococcus epidermidis	Listeria monocytogens	3mm

KEYS: MS= Meat Sample,Significant activity starts from 2mm and above.

The isolates were screened for the determination of bacteriocin activity and quantification of bacteria at 37^0C for 48hours. Lactobacillus acidophilus (MSI) at concentration of 35Au/ml. Bacillus cereus (MS2) at concentration of 27Au/ml and Staphylococcus epidermidis (MS3) at concentration of 23Au/ml were able to produce bacteriocins (Fig 2).The isolates were screened for activity/qualification of the bacterial isolates at 37^oC for 48hrs. The absorbance of the isolates with higher colony counts was taken and the concentration of the bacteriocin was gotten when compared against the standard curve chart. Lactobacillus acidophilus (MS1) at concentration of 35Au/ml, Staphylococcus epidermidis (MS2) at concentration of 23Au/ml and Bacillus cereus at concentration of 27Au/ml would be able to produce bacteriocin.

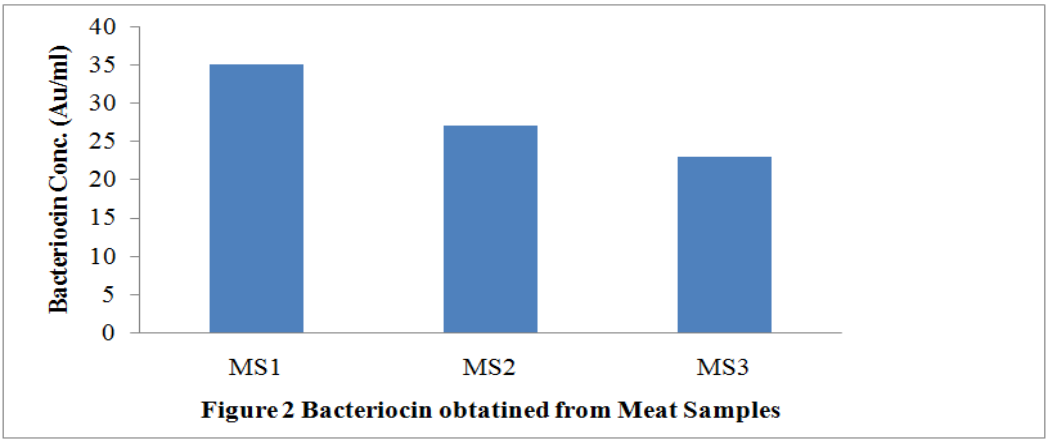

Figure 2 Bacteriocin obtatined from Meat Samples

KEY: MS= Meat Sample, Au = Arbitrary unit

According to Naclerioet al., (1993), Cereins are a group of bacteriocins produced by various strains of the bacterium Bacillus cereus. Although, all cereins are by definition produced by B. cereus it is possible that they are chemically quite different from one another. Cereins have been found to be active against other strains of B. cereus as well as a broad range of other gram positive bacteria.

IV. Conclusion

In conclusion, Lactobacillus acidophilus, Bacillus cereus and Staphylococcus epidermidis are good bacteriocin producing bacteria and are effective on the biopreservation of food pathogens. Bacteriocins from Lactobacillus acidophilus were highly effective against Salmonella typhi, Escherichia coliandListeria monocytogenes while Bacillus cereus and Staphylococcus epidermidis was effective against Brochothrixthermosphacta and Listeria monocytogenesrespectively. The colonies observed from Lactobacillus

acidophilus was higher (1.8 x 10^5cfu/g) when compared to those from Bacillus cereus and Staphylococcus epidermidis with colony counts of 1.6 x 10^5cfu/g and 1.5 x 10^5cfu/g respectively**.** Further studies are recommended to assess the proper dose and administration route of these biopreservatives for commercial purpose.

References

[1]. Arques JL, Fernandez J, Gaya P, Nunez M, Rodriguez E, Medina M, 2004.Antimicrobial activity of reuterin in combination with nisin against food-borne pathogens. Int J Food Microbiol, 95:225-229.
[2]. Bedford, M. 2000. Removal of antibiotic growth promoters from poultry diets: implications and strategies to minimize subsequent problems. World's Poultry Science Journal 56: 347–365.
[3]. Bouttefroy A, Milliere JB, 2000. Nisin-curvaticin 13 combinations for avoiding the regrowth of bacteriocin resistant cells of Listeria monocytogenes ATCC 15313.Int J Food Microbiol, 62:65-75.
[4]. Cheesbrough, M, 2006. District Laboratory Practice in Tropical Countries. 2nd Edn., Cambridge University Press, Cambridge, UK.
[5]. Cleveland J, Montville TJ, Nes IF, Chikindas ML, 2001. Bacteriocins: safe, natural antimicrobials for food preservation. Int J Food Microbiol, 71:1-20.
[6]. Fawole, M.O. and Oso B.A. , 2004. Characterization of Bacteria: Laboratory Manual of Microbiology. 4thEdn, Spectrum Book Ltd, Ibadan, Nigeria, pp. 24-33
[7]. Franz CM, Van Belkum MJ, Holzapeel WH, Abriouel H, Galvez A, 2007. Diversity of enterococcalbacteriocins and their grouping in a new classification scheme. FEMS Microbiol Rev, 31:293-310.
[8]. Javed A, Masud T, Ain QU, Imran M, Maqsood S, 2011. Enterocins of Enterococcus faecium, emerging natural food preservatives.Ann Microbiol, 61:699-708.
[9]. Joerger, RD. (2003) Alternatives to Antibiotics, Bacteriocins, Antimicrobial Peptides and Bacteriophages. Poultry Sci. 82, 640–647.
[10]. Kapil, A. (2005), The challenge of antibiotic resistance: need to contemplate. Indian J. Med. Res. 121, 83-91.
[11]. Kellner R, Jung G , Horner T, Zahner H, Schnell N, Entian KD , Gotz F (1988). Gallidermin: a new lanthionine- containing polypeptide antibiotic. Eur. J. Biochem. 177(1):53-9.
[12]. Mantovani, H.C., Cruz, A.M.O. and Paiva, A.D. 2011. Bacteriocin activity and resistance in livestock pathogens. Méndez-Vilas (Ed.) FORMATEX, 853863.
[13]. Olutiola, P.O., O. Famurewa and H.G.Sonntag, 2000. Introduction to General Microbiology: A practical Approach .2ndEdn, Bolabay Publication, Ikeja, Nigeria.
[14]. Todorov, S.D. and L.M.T. Dicks, 2004.Comparison of 2 methods for purification of plantaricin ST31, a bacteriocin produced by Lactobacillus plantarium ST31.Enzyme Microbial. Technol, 36:183-187.
[15]. Jadamus, A, Vahjen, W, Schafer, K and Simon,O (2002) Animal Nutrition. Journal of Animal Physiology, 86: 42–54.
[16]. K. H. Lee, K. D. Jun, W. S. Kim, H. D. Paik 2001. Lett. Appl. Microbiol. 32: 146–151
[17]. Coventry, M. J.; Gordon, J. B.; Alexander, M.; Hickey, M. W. and Wan, J. (1996), A food-grade process for isolation and partial purification of bacteriocins of lactic acid bacteria that uses diatomite calcium silicate. Appl. Environ. Microbiol., 62, 17641769.
[18]. Moreno, I.; Lerayer, A S. L.; Baldini, V. L. S. and Leitão, M. F. de F. (2000), Characterization of bacteriocins produced by Lactococcuslactis strains. Braz. J. Microbiol., 31, 184-192.
[19]. Naclerio, G., Ricca , E; Sacco, M; De Felice, M(December 1993). "Antimicrobial activity of a newly identified bacteriocin of Bacillus cereus ".Appl Environ Microbiol. 59(12): 4313-6.
[20]. Scallan E, Griffin PM, Angulo FJ, Tauxe RV, Hoekstra RM, 2011. Foodborne illness acquired in the United States-unspecified agents.Emerg Infect Dis, 17:16-22.
[21]. Turgis M, Vu KD, Dupont C, Lacroix M, 2012. Combined antimicrobial effect of essential oils and bacteriocins against foodborne pathogens and food spoilage bacteria.Food Res Inter, 48: 696-702.
[22]. Sunde M, Nordstrom M, 2006. The prevalence of, associations between and conjugal -transfer of antibiotic resistance genes in Escherichia coli isolated from Norwegian meat and meat products. J AntimicrobChemother, 58:741-747.
[23]. Thomas LV, Delves-Broughton J., 2005. Nisin. In: Davidson PM, Sofos JN, Branen AL, editors. Antimicrobials in food. Boca Raton, FL, USA: CRC Press. Pp. 237–74.
[24]. Van Kraaij C, de Vos WM, Siezen RJ, KuipersOP (October 1999). "Lqntibiotics biosynthesis, mode of action and applications''.Nat Prod. Rep 16(5): 575-87
[25]. Walsh C, Fanning S, 2008. Antimicrobial resistance in foodborne pathogens-a cause for concern?Curr Drug Targets, 9:808-815.
[26]. Wan,J., Hickey, M.W.& Coventry, M.J.1995. Continous production of bacteriocins ,brevicin, nisin and pediocin, using calcium alginate-immobilized bacteria. J. Appl. Bacteriol . 79, 671-676.

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