



SHORT COMMUNICATION

Histopathological studies on the effect of bacteriocin producing *Bacillus cereus* isolate from 'wara' a local soft cheese on the liver, kidney and reproductive organs of Wistar albino rats

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Aims: This study was done to generate a baseline data on the effect of *Bacillus cereus* and its bacteriocin on the liver, kidney and reproductive organs in both sexes at different concentration.

Methodology and results: *Bacillus cereus* and its bacteriocin were injected intramuscularly in male and female Wistar rats at doses equivalent to 10^2 CFU and 10^4 CFU dilutions. Body weights were also noted. The liver, kidney and reproductive organs of the animals were examined for histopathological changes. The liver of female rats administered *B. cereus* at 10^2 CFU showed portal and cellular infiltration by mononuclear cells, diffuse hydropic degeneration and severe interstitial hemorrhages of the kidney was observed when 10^4 CFU of *B. cereus* was given. Male rats administered 10^2 CFU and 10^4 CFU of *Bacillus cereus* showed diffuse hydropic degeneration and portal congestion of the liver while at 10^4 CFU the kidney showed diffuse, moderate interstitial cellular infiltration. This is more evident in the wistar rats administered with *bacilli* organism than the groups that received the bacteriocin. The reproductive organs of treated animals showed no pathological lesions. There were no visible tissue pathological changes in the untreated groups. There were no visible tissue pathological changes in the untreated groups.

Conclusion, significance and impact study: The absence of observable toxic effects of the bacteriocin of *B. cereus* on the sex organs, is not sufficient to determine the safety of this bacteriocin since pathological lesions were observed in the liver and kidney. We hereby suggest a further study on characterization and purification of this bacteriocin as a biopreservative in items not meant for human use or consumption.

Keywords: toxicity, *Bacillus cereus*, bacteriocin, rat, liver, kidney.

INTRODUCTION

Bacillus is an interesting genus to investigate since it produces a diverse array of antimicrobial peptides representing several different basic chemical structures (Bizani and Brandelli, 2002). The production of bacteriocins or bacteriocin-like substance has already been described for some Bacilli such as *B. subtilis*, *B. cereus*, *B. stearothermophilus*, and other *Bacillus* species. Some strains produce bacteriocins with a broad spectrum activity, including important pathogens such as *Listeria monocytogenes* and *Streptococcus pyogenes* (Cherif *et al.*, 2001). Some were well characterized such as lichenin produced by *B. licheniformis* 26-103 RA strain (Pattnaik *et al.*, 2001) and megacin produced by *B. megaterium* (Lisboa *et al.*, 2006).

Bacillus cereus is one of around 60 represent actives of the widely varied *Bacillus* genus along with the very similar species *B. mycoides*, *B. thuringiensis* and *B. anthracis*. It comprises the so called "*Bacillus cereus*"

group. The differences between these four species are very small. *B. cereus* is found frequently as a saprophyte in soil water, vegetation and air, from where it is easily transferred to food, either from the original raw material, or during the food processing. It is common in dried foodstuffs spices, cereals, meat, egg, milk and milk products, cooked, and inappropriately kept food (Kramer *et al.*, 1989; Becker *et al.*, 1994; Notermans *et al.*, 1997). *B. cereus* closely related species from the genus *Bacillus* have several features including the production of various biologically active metabolites ie. antibiotics, proteinases and bacteriocins that make them attractive candidates for biological control agents. It is well known that most, if not all bacteria species are capable of producing a heterogeneous array of molecules in the course of their growth *in vitro* (and presumably also in their natural habitats) and may be inhibitory to other bacteria (Tagg *et al.*, 1976).

In spite of the introduction of modern technologies and safety concepts, the reported number of food-borne

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illnesses and intoxications are on the rise. Many of the ready-to-eat and novel food products represent new food systems with respect to health and spoilage risks. In the light of the above as well as improved understanding and knowledge of the complex microbial interactions, there is an increased tendency to use biopreservatives in the form of protective cultures or their metabolites, ie. enzymes and bacteriocins (Holzapfel *et al.*, 1995). Biological presentation which is a scientific approach to improve the microbial safety of foods refers to the use of antagonistic microorganism or their metabolic products to inhibit or destroy undesirable microorganisms in foods. Lactic acid bacteria (LAB) have been exploited for the production of fermented foods due to their ability to produce desirable changes in the taste, flavor and texture as well as inhibit pathogenic and spoilage microorganisms. Since they are prevalent in numerous fermented foods, it is assumed that most representatives of this group do not pose any health risk to man, and are designated as GRAS (Generally Recognized as Safe) organisms. The LAB, generally considered as 'food grade' organisms, has a special promise as protective cultures (Holzapfel *et al.*, 1995).

In attempt to control pathogenic bacteria in food, the production of antimicrobial peptides from bacteria "bacteriocins" has been given consideration. Bacteriocins are antimicrobial proteins or oligopeptides that are active against bacteria strains closely related to the producer strain (Jung, 1991). They are heterogeneous compounds which vary in molecular weight, biochemical properties, activity spectra and mechanism of action (Cheriff, 2001). These polypeptide antibiotics can possess bactericidal, fungicidal, metal chelating and immunomodulation activities. They are frequently found in secondary metabolites produced by various microorganisms, such as Gram positives bacteria of the genus *Streptomyces*, lactic acid bacteria and genus *Bacillus* (Katz, 1977). Some bacteriocins kill only bacteria belonging to the same species as producer whereas other bacteriocins kill a broad range of Gram positive bacteria (Conventry *et al.*, 1997; Ennahar *et al.*, 2000). The incorporation of these compounds as biopreservative ingredient into model food has been shown to be effective in the control of pathogenic and spoilage micro-organisms (O'Sullivan *et al.*, 2002).

In this present study, the bacteriocin of *B. cereus* appeared to be safer as a biopreservative when compared to its *bacillus* organism. However, further studies are needed to ascertain the safety of this bacteriocin in biopreservation in items not meant for human use.

MATERIALS AND METHODS

Bacilli strain

B. cereus was harvested from 'wara' a West African soft cheese and was selected as potential bacteriocin producer and identified on the basis of its cultural,

morphological, physiological and biochemical characteristics (Barrow and Feltham, 1993).

Test animals

Male and female albino rats, *Rattus norvegicus* albinos, aged 9-10 weeks old weighing 90-120 g was purchased from the animals' house, University of Ibadan, Ibadan, Nigeria. Rats were kept under the laboratory conditions of 25±5 °C and 65±5% R.H, three weeks to stabilize. They were housed in metal cages (25×20×15 cm) and maintained on pelleted growers mash (protein: 21% min, fat: 3.5% min, fibre: 6.0% max, calcium: 0.8%) from Ladokun feeds Ltd. Ibadan, Nigeria and also on water. Animal experiments and housing procedures were performed in accordance to the animal care rules and they were approved by the animal welfare committee of the University.

Preparation of inoculums

B. cereus was stored in glycerol at -20 °C and was purified by sub culturing of the colonies in plated media and incubated at 30-37 °C for 24 h. In an effort to obtain a pure strain, a discrete microbial colony was picked with the aid of a sterile wire loop. This was streaked out on a new nutrient agar plate and incubated at 37 °C. The sub-culturing was done three times to obtain a pure colony. The concentration of *B. cereus* and its bacteriocin that was inoculated into the rats were determined by a 10 fold serial dilution of the 1 mL broth culture. 0.1 mL of the sample strain was inoculated into a Petri dish containing prepared nutrient agar and incubated at 30-37 °C for 24 h which gave a total Colony Forming Unit (CFU) of 5×10¹⁴ CFU/mL.

Detection of antimicrobial activity by Agar Well Diffusion assay (AWD)

Nutrient agar plates seeded with *Micrococcus luteus* was used for the agar well diffusion test. A 5 mm diameter wells were created with a sterile club. The wells were then layered with viscous nutrient agar. 10² CFU/mL of *B. cereus* was placed in wells and incubated at 37 °C for 24 h, the diameter of the zone of growth inhibition were then measured in mm.

Harvesting of bacteriocin

B. cereus was grown in 10 mL of nutrient broth and incubated at 37 °C for 18-24 h. The broth was centrifuged at 3500 revolution for 15-20 min after which the bacteriocin was drawn out into another test tube using a pipette to prevent mixing with the bacteria cells below the test tubes. The supernatant was decanted into sterile test tubes, adjusted to pH 6.5-7.0 with NaOH (40 g/1000 mL) to remove organic acid effect. H₂O₂ was neutralized by addition of catalase from bovine liver at 200 µg/mL. The mixture of the supernatant of culture, NaOH and catalase was filtered and sterilized with a 0.2 µm Millipore filter

membrane. This filtrate (bacteriocin) was covered with foil paper and then stored at 4 °C to prevent contamination until use.

Acute toxicity study

Ten albino rats were divided into five groups of two animals each. Each group has male and female rat with their replicates. The first and second groups were treated with *B. cereus* at doses equivalent to 10² CFU and 10⁴ CFU dilutions while the third and fourth groups were given the bacteriocin of *B. cereus* at doses equivalent to 10² CFU and 10⁴ CFU dilutions. The fifth group which was kept as control received distilled water. The intramuscular route was used in all the groups. All the animals were observed for clinical signs and mortality for 15 days. Feed and water was administered *ad libitum*. The body weight of the rats were taken before the commencement of the work and on day 15. The animals were sacrificed with use

of cervical dislocation and the liver, kidney, testes and ovaries of all the animals were harvested and fixed in 10% buffered formalin in labeled bottles. Tissues were processed routinely and embedded in paraffin wax. Section 5 microns thick were cut stained with haematoxylin and eosin and examined under light microscope according to (Lillie and Fullmen, 1976).

RESULTS AND DISCUSSIONS

Clinical findings

Male rat treated with *B. cereus* at 10⁴ CFU, female rat given *B. cereus* at 10² CFU and 10⁴ CFU died three hours after the collection of blood samples at day 10. There were no significant changes in weight of wistar rats before and after administration of the pathogen and its bacteriocins (Table 1).

Table 1: Weights(g) of rat before and after inoculation of *Bacillus cereus* (B) and its bacteriocin.

	FOB ²	MOB ²	FOB ⁴	MOB ⁴	FbB ²	MbB ²	FbB ⁴	MbB ⁴	FCO	MCO	FCb	MCb
Before	120	80	80	80	80	80	80	120	80	90	80	80
After	125	81	80	-	-	82	80	118	80	90	80	80

FOB²: female rat given *Bacillus* organism at 10² CFU
 MOB²: male rat given *Bacillus* organism at 10² CFU
 FOB⁴: female rat given *Bacillus* organism at 10⁴ CFU
 MOB⁴: male rat given *Bacillus* organism at 10⁴ CFU
 FbB²: female rat given bacteriocin at 10²CFU
 MbB²: male rat given bacteriocin at 10² CFU

FbB⁴: female rat given bacteriocin at 10⁴ CFU
 MbB⁴: male rat given bacteriocin at 10⁴ CFU
 FCO: female rat used as control for the *Bacillus* organism
 MCO: male rat used as control for the *Bacillus* organism
 FCb: female rat used as control for the bacteriocin
 MCb: male rat used as control for the bacteriocin

Histopathological findings

The liver of female rats administered *B. cereus* at 10² CFU showed portal and cellular infiltration by mononuclear cells (Figure 1), diffuse hydropic degeneration at 10⁴ CFU (Figure 2) and severe interstitial hemorrhages of the kidney (Figure 3) when 10⁴ CFU of *B. cereus* was given. Male rats administered 10² CFU and 10⁴ CFU of *B. cereus* showed diffuse hydropic degeneration and portal congestion of the liver (Figures 4 and 5) while at 10⁴ CFU the kidney showed diffuse, moderate interstitial cellular infiltration (Figure 6). No pathologic changes were observed in the internal organs of male and female rats administered bacteriocin of *Bacillus cereus* at 10² CFU and 10⁴ CFU concentrations. However, the reproductive organs of both sex showed no pathological lesions.

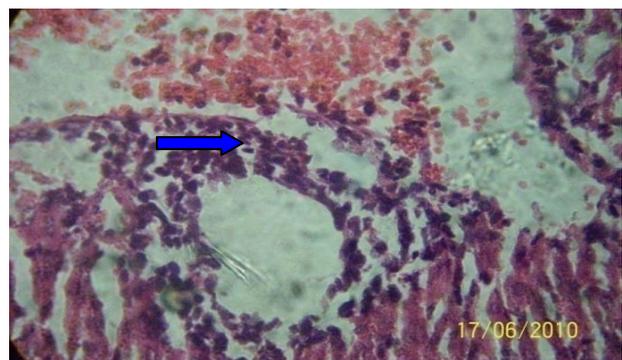


Figure 1: Liver from female rat that received 10² CFU *B. cereus*.
 Note: portal and diffuse cellular infiltration by mononuclear cells (H & E x 400).

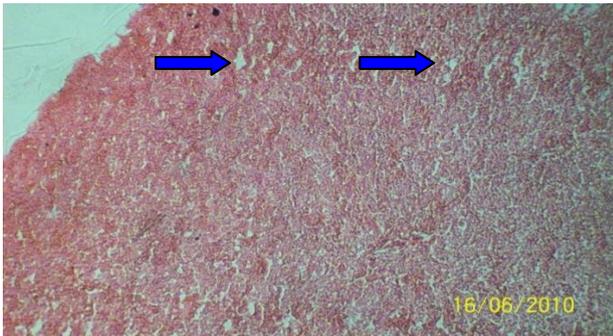


Figure 2: Liver from female rat that received 10^4 CFU *B. cereus*.
Note: diffuse hydropic degeneration (H&E 400).

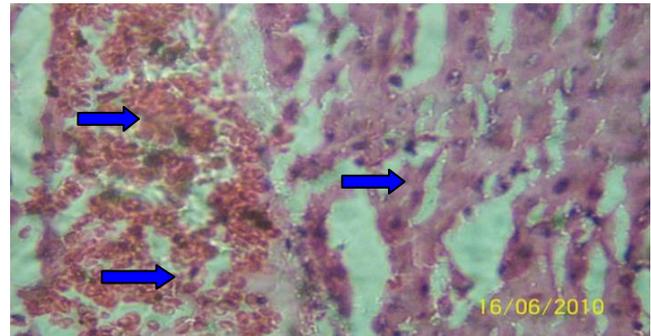


Figure 5: Liver from male rat that received 10^4 CFU *B. cereus*.
Note: moderate portal congestion (H & E x 400).

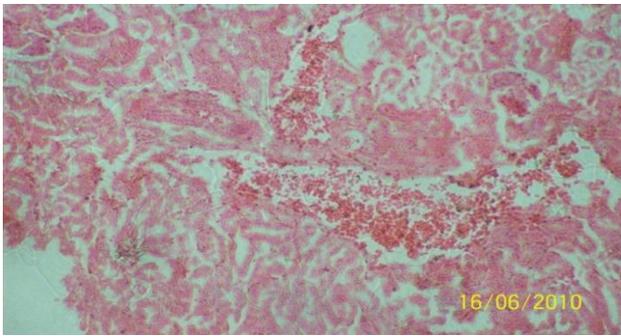


Figure 3: Kidney from female rat that received 10^4 CFU *B. cereus*.
Note: severe interstitial hemorrhages (H & E x 100).

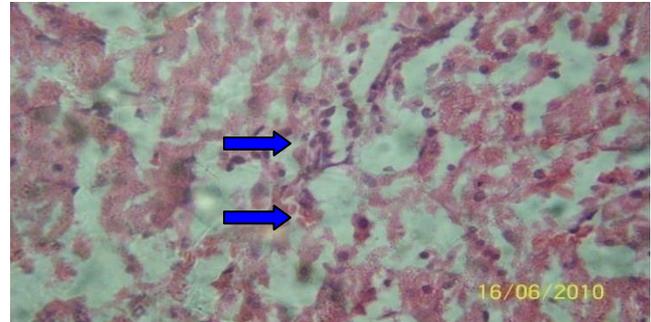


Figure 6: Kidney from male rat that received 10^4 CFU *B. cereus*.
Note: Diffuse, moderate interstitial cellular infiltration.

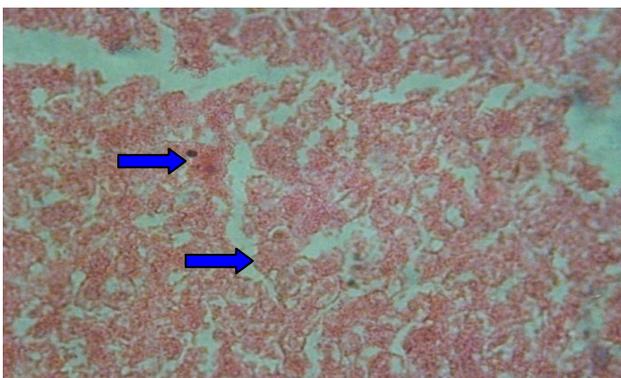


Figure 4: Liver from male rat that received 10^2 CFU *B. cereus*.
Note: Diffuse hydropic degeneration and portal congestion (H & E x 100).

Liver is often the primary target of the toxic effects of materials. It is known that the detoxification of the toxic materials which enter the body occurs mainly in the liver (Balistreri and Shaw, 1987). Infiltration by mononuclear cells in the liver and kidney tissues is due a response to injurious changes. A similar report of cellular infiltration in the liver tissue post administration was postulated that such changes were a prominent response of body tissue facing any injurious impacts (El-Banhawy *et al.*, 1993). Hydropic degeneration which is a mild form of cell sickness could be associated with acute toxic injury though could be reversible. Congestions which are observed could also be as a result of the cellular infiltration due to the presence of *bacillus cereus* in the system.

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