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ANTIBACTERIAL EFFECT OF BACTERIOCIN FROM *LEUCONOSTOC MESENTEROIDES* SSP. *CREMORIS* AGAINST DIARRHEAL CAUSATIVE BACTERIA

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ABSTRACT
Antibacterial activity of crude bacteriocin of *Leuconostoc mesenteroides ssp. cremoris* included non-concentrated and concentrated supernatant (bacteriocin) was tested against diarrheal causative bacteria *Escherichia coli, Salmonella ser. paratyphi A, Shigella group.* and *Vibrio cholerae*. Results showed that non-concentrated crude supernatant (bacteriocin) from 2 isolates [*L. mesenteroides* ssp. *cremoris* (Lc3) and *L. mesenteroides* ssp. *cremoris* (Lc4)] had inhibitory effect with inhibition diameter ranged between (11-13) mm against *E.coli, Shigella group, Salmonella ser. paratyphi A* and *V. cholera*, while other isolates were unable to inhibit the growth of pathogenic bacteria. The concentrated crude supernatant bacteriocin showed inhibitory activity against *E.coli, Shigella group, Salmonella ser. paratyphi A* and *V. cholerae* with diameter of 19, 18, 15 and 25 mm respectively for for *Leuconostoc mesenteroides* ssp. *cremoris* (Lc3) isolate and inhibition diameter of 17, 18, 16 and 20 mm for *Leuconostoc mesenteroides* ssp. *cremoris* (Lc4) isolate.

KEYWORDS: *Leuconostoc mesenteroides,*Bacteriocin, Diarrheal causative bacteria.

INTRODUCTION
Leuconostoc mesenteroides ssp. cremoris characterized as gram positive coccobacilli bacteria, they are non-motile, catalase- negative and non- spore former bacteria, they are found in foods of animal origin which include raw milk, dairy products, poultry, meat and fish as well as found in vegetable products, despite the resistance to vancomycin it is sensitive to erythromycin and clindamycin (Usta-Atmaca *et al.,* 2015).

It is a part of the lactic acid bacteria, it`s able to produce anti molecules such as organic acids, hydrogen peroxide, a dual acetylcholine, and bacteriocins (Ringø *et al.*, 2010).

Bacteriocins are antimicrobial peptides (AMPs) which contains molecules acts as a defense system against many organisms, such as bacteria, fungi, parasites and viruses. These components become very important due to the increase in microorganisms resistant to the usual vital antibiotics (Balciunas *et al.*, 2013).

Diarrhea is a major cause of morbidity among children in developing countries. transmitted microbes responsible for the majority of injuries through the feces to the mouth through water and food (Fung *et al.*, 2011). It is caused by a wide range of pathogens, including bacteria, viruses, etc, only a small number of organisms are the cause of most cases of acute childhood diarrhea such as adinovirus and rotavirus reasons leading to acute diarrhea, The biggest section contains pathogenic bacteria such as *E.coli, Shigella,, Campylobacter, Salmonella, V.cholera Clostridium difficili* caused diarrhea during epidemics (Moreno *et al.*, 2000 ؛Hera *et al.*, 2016).

The mechanism of diarrhea occurrence are adhesion of pathogenic bacteria to the small intestine and the production of toxins when they connected to particularly receptors located on the cell membrane layer of the gut where it lead to activate the enzyme adenylate cyclase where increasing its concentration leads to increasing Cycli Adenosin Mono phosphate (cAMP) and thus lead to increased fluid loss and salts from the intestine, causing diarrhea (Ateia, 2013).

This study was aimed to production of bacteriocin from the *Leuconostoc mesenteroides ssp. cremoris* isolated from raw cow milk and study their antibacterial effect against some bacteria causing diarrhea.

MATERIALS AND METHODS

Bacterial Isolates

Isolation and Identification of *Leuconostoc mesenteroides*

For *Leuconostoc mesenteroides* isolation, each sample of raw cow milk was inoculated in MRS broth at (1%) and cultivated at 30°C for 24 h under anaerobic condition. A series of dilutions (10^{-1},10^{-2}, 10^{-3},10^{-4}) were made for each sample with phosphate buffer saline, then spread (0.1) ml of the fourth dilution on solid MRS Vancomycin agar plates (Fatma and Benmechernene,2013). MRS Vancomycin agar plates were incubated at 30°C for 24 h anaerobically. Sub culturing of isolated colonies on MRS agar for further purification, incubated under anaerobic condition at 30°C for 24 h, then the individual colonies were transported to MRS broth and incubated under the same conditions. The purified isolates were identified through cultural, microscopically and biochemical tests (Gravie, 1986) and Vitek2 system.

Isolation and Identification of diarrheal causative bacteria

The diarrheal causative bacteria was isolated from feces of children under five years old and it was identified through cultural, microscopically and biochemical tests and Vitek2 system according to (Baron and Finegold, 1994).

Detection of bacteriocin production by *Leuconostoc mesenteroides* ssp. *cremoris*

The bacterial isolates were inoculated in MRS broth with ph. 6.2 for 24h at 37℃. The crude supernatant(bacteriocin) was obtained by centerifugation at 10,000 rpm for 15 min, sterilized by Millipore filter (0.02 m), the supernatant was neutralized to ph. 6.2 by adding 1N NaOH to remove the influence of organic acid (Salman, 2014).

Concentration of crude supernatant (bacteriocin)

The crude bacteriocin was concentrated using Dialysis bags and poly ethylene glycol 20000 (Janson and Ryden., 1998).

Antibacterial effect of crude bacteriocin from *L. mesenteroides* ssp. *cremoris*

Leuconostoc mesenteroides ssp. cremoris non concentrated and concentrated crude supernatants (bacteriocin) were screened for their antibacterial activity against *Escherichia coli*, *Salmonella spp.*, *Shigella spp.* and *Vibrio cholerae* isolates, using agar well diffusion method. The plates were spread with 10^5cfu/ml culture broth of pathogenic isolates on the surface of nutrient agar, then the plates were left for 10 min dispensing 50µl of each samples into the wells. The plates were incubated at 37℃ for 24h. Inhibition zone were measured in millimeter diameter (Gupta, 1996).

RESULTS AND DISCUSSION

Isolation and Identification of *Leuconostoc mesenteroides*

Twelve isolates of *L. mesenteroides* were obtained from 25 bacterial isolates. All isolates were Gram positive, ovoid shape associated in pairs and/or short chains, negative catalase reaction, able to produce carbon dioxide from glucose and unable to hydrolyze Arginine. All isolates were grown at 45°C. After identification by biochemical tests and Vitek 2 system, the results showed that 9 out of 12 isolates belonged to *Leuconostoc mesenteroides* ssp. *cremoris*, while 2 isolates of *Leuconostoc mesenteroides* ssp. *mesenteroides* and one isolate *Leuconostoc mesenteroides* ssp. *dextranicum* (Table-1-).

Table(1): Incidence of *Leuconostoc* isolates from raw milk.

Type of isolates	Number of isolates
Leuconostoc mesenteroides ssp. *cremoris*	9
Leuconostoc mesenteroides ssp. *mesenteroides*	2
Leuconostoc mesenteroides ssp. *dextranicum*	1
Total	12

From the result above, 9 of 12 isolates were identified as *L.mesenteroides* ssp. *cremoris* which isolated from raw milk, Abbas (2015) reported that the majority of *Leuconostoc* spp. isolated from raw milk were identified as *L.mesenteroides* ssp. *cremoris*, while Fatma and Benmechernene (2013) reported the possibility to isolate *Leuconostoc* spp. from raw camel milk. Bao *et al.*(2012) mentioned that 40.8% of lactic acid bacteria isolated from raw milk product was belonged to *Leuconostoc* spp.

In this research 15 isolates of diarrheal causative bacteria were identified as (9) *E.coli*, (2)*Salmonella ser. paratyphi A*, (3)*Shigella group* and one isolate of *Vibrio cholerae*,

Detection of bacteriocin production by *Leuconostoc mesenteroides ssp. cremoris*

Screening of crude supernatant (bacteriocin) of *L. mesenteroides* ssp. *cremoris* isolates to select the more efficient producing bacteriocin isolates, the results showed that *L. mesenteroides* ssp. *cremoris* (Lc3) and *L. mesenteroides* ssp. *cremoris* (Lc4) isolates gave the highest inhibition activity against *E.coli* and *Shigella group* with inhibition diameter (10-13) mm, against *Salmonella ser. paratyphi A* (10-12) mm, while against *Vibrio cholerae* was (11-12) mm (Table-2-). Gupta *et al.*, (1998) observed that the supernatant of *lactobacillus* bacteria grown on MRS broth gave inhibitory activity against gram positive and negative bacteria, Salman *et al.*, (2008) noticed the inhibitory activity of of the supernatant of lactic acid bacteria against bacteria causing UTI urinary tract infection, Karthikeyan and Santhoth, (2009) showed that MRS broth is the best medium for the production of Bacteriocin, O`Shea *et al*., (2011) mentioned that the inhibitory activity of supernatant of *Lactobacillus salivarius* was limited effect, Salman, (2014) showed that *Staphylococcus aureus* (PSM producer isolates) showed resistance to the neutralized concentrated cell free supernatant (CSF) and bacteriocin of *Lactobacillus salivarius*.

Table(2): Antibacterial activity of *Leuconostoc mesenteroides* ssp. *cremoris* crude supernatant (bacteriocin) against diarrheal causative bacteria.

Pathogenic bacteria isolates	Inhibition zone (mm)								
	Lc1	Lc2	Lc3	Lc4	Lc5	Lc6	Lc7	Lc8	Lc9
E.coli (E1)	-	-	10	10	-	-	-	-	-
E.coli (E2)	-	-	11	13	-	-	-	-	-
E.coli (E3)	-	-	12	12	-	-	-	-	-
E.coli (E4)	-	-	11	11	-	-	-	-	-
E.coli (E5)	-	-	13	12	-	-	-	-	-
E.coli (E6)	-	-	11	10	-	-	-	-	-
E.coli (E7)	-	-	12	13	-	-	-	-	-
E.coli (E8)	-	-	12	12	-	-	-	-	-
E.coli (E9)	-	-	11	11	-	-	-	-	-
Shigella group (Sh1)	-	-	10	12	-	-	-	-	-
Shigella group (Sh2)	-	-	12	11	-	-	-	-	-
Shigella group (Sh3)	-	-	11	12	-	-	-	-	-
Salmonella ser. paratyphi A (Sa1)	-	-	12	12	-	-	-	-	-
Salmonella ser. paratyphi A (Sa2)	-	-	11	12	-	-	-	-	-
Vibrio cholerae (V1)	-	-	12	11	-	-	-	-	-

Lc: *Leuconostoc mesenteroides* ssp. *cremoris* , - : no inhibitory effect

Inhibitory effect of concentrated crude supernatant (bacteriocin) against diarrheal causative bacteria.

The concentrated crude supernatant (bacteriocin) produced by *L.mesenteroides* ssp. *cremoris* showed inhibitory effect against all pathogenic bacteria with inhibition zone ranged from (12-19) mm against *E.coli*, (14-18) mm against *Shigella group*, (14-15) mm *Salmonella ser*, *paratyphi A* and (22) mm against *Vibrio cholera* for *L. mesenteroides* ssp. *cremoris* (Lc3) isolate and from (11-17) mm against *E.coli*, (16-18) mm against *Shigella group*, (15-16) mm against *Salmonella ser*, *paratyphi A* and (20) mm against *Vibrio cholera* for *L. mesenteroides* ssp. *cremoris* (Lc4) isolate. (Table-3-) (Figure 1, 2).

The reason for the effectiveness of inhibitory activity of concentrated crude supernatant against many gram positive and negative bacteria due to the ability of bacteria to produce many inhibitory substances, especially bacteriocins (Brashear *et al*., 2003), Salman (2014) observed that the concentrated supernatant of lactic acid bacteria showed inhibitory activity against *S. aureus*.

The impact of bacteriocin in bacterial cells can be either fatal bactericidal (Todorov, 2009; Al-Charrakh et al., 2011) or it can be a disincentive to the growth bacteriostatic (Serika et al., 2010) and similar mechanisms of action of bacteriocins on its impact on the target cell with the mechanisms of action of antibiotics it has been acting on cell wall or plasma membrane (Han et al., 2011). Sarika et al., (2010) have showed that bacteriocins produced from *lactobacillus rhamnosus* had an effective inhibitory against *E.coli* , *S. aureus*, *Pseudomonas aeruginosa* and *Vibrio harveyi*.

Table(3): Antibacterial activity of *Leuconostoc mesenteroides* ssp. *cremoris* concentrated crude supernant (bacteriocin) against diarrheal causative bacteria.

Pathogenic bacteria isolates	Inhibition zone (mm)	
	***Leuconostoc mesenteroides* ssp. *cremoris* (Lc3)**	***Leuconostoc mesenteroides* ssp. *cremoris* (Lc4)**
E.coli (E1)	12	11
E.coli (E2)	13	16
E.coli (E3)	18	16
E.coli (E4)	18	17
E.coli (E5)	19	16
E.coli (E6)	12	12
E.coli (E7)	18	15
E.coli (E8)	19	16

E.coli (E9)	14	14
Shigella group (Sh1)	14	17
Shigella group (Sh2)	18	18
Shigella group(Sh3)	14	16
Salmonella ser. paratyphi A (Sa1)	15	15
Salmonella ser. paratyphi A (Sa2)	14	16
Vibrio cholerae (V1)	25	20

Lc: *Leuconostoc mesenteroides* ssp. *cremoris* , - : no inhibitory effect

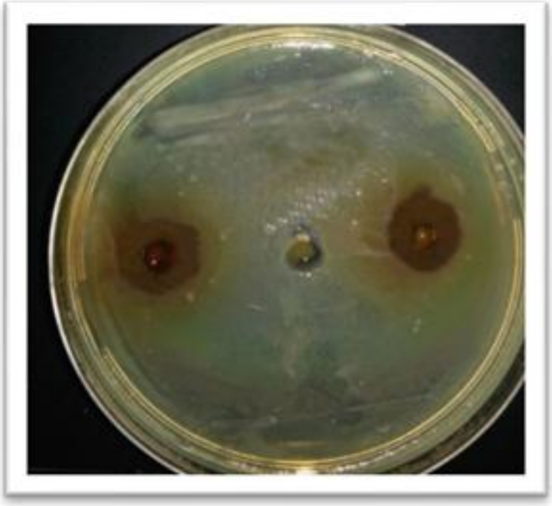

Figure (1): Antibacterial activity of Leuconostoc mesenteroides ssp. cremoris concentrated crude supernant (bacteriocin) against E.coli.

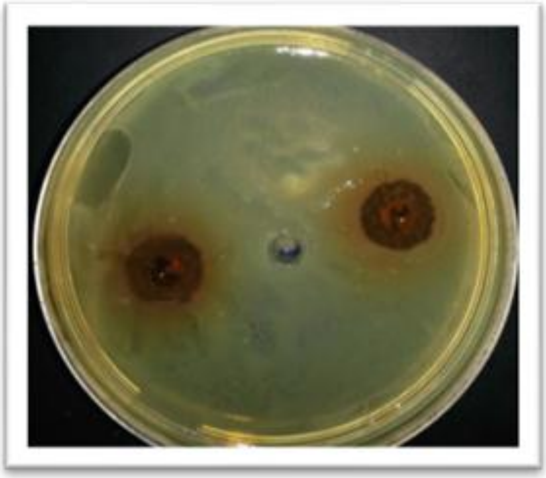

Figure (2): Antibacterial activity of Leuconostoc mesenteroides ssp. cremoris concentrated crude supernant (bacteriocin) against Shigella group.

CONCLUSION

In conclusion, some of Leuconostoc mesenteroides ssp. cremoris isolated from local raw cow milk had ability to produce bactriocin that inhibit diarrheal causative bacteria.

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