

Antipathic and Non Tenacious Effect of Bacteriocin Producing Strains against Pathogenic Organisms

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ABSTRACT

Bacteriocin is the proteinaceous compounds synthesized from the ribosomes that serve as the site of biological protein synthesis. It is the biological active compound that has salubrious effects against pathogenic species. Both gram negative and gram positive bacteria have ability to produce these proteinaceous compounds. In the present study, isolates were collected from different sources such as Lactobacillus (from raw milk, cheese, yogurt and dry milk), E.coli (from sewage sample), Pseudomonas and Bacillus (from soil). The bacteriocin production was done by inoculating the isolated colonies of these organisms into their respective broths. After that, estimation of the bacteriocin production was done by measuring their optical density. Antimicrobial activity of bacteriocin was conducted by both Agar well diffusion and over lay technique against pathogenic strains. In agar well diffusion technique the antibacterial strength of the bacteriocin may be judged by the width of the zone of inhibition around it. Bacteriocin isolated from Lactobacillus yogurt sample gives 34.56% clear zone of inhibitions, 33.82% was from Lactobacillus raw milk sample, 18.76% from Pseudomonas, 6.66% from Bacillus and 6.17% zone of inhibition was given by E.coli. The results obtained by over lay technique reveals the less turbidity on the plate or inhibition of bacterial growth will cause a reduction in the turbidity of the lawn near the bacteriocin seeded area. This study reveals that this proteinaceous compound have lethal effect on the disease causing organisms. Although these organisms have anti-adhesive property. These antagonistic activities of bacteriocin can be used in a research tool as a beneficial point of view to produce antibiotics or other pharmaceuticals products to reduce infectious disease cause by pathogenic organisms.

Keywords: Bacteriocin, Antagonistic mechanisms, Pharmaceuticals, Anti adhesive, Inhibition.

INTRODUCTION

Antibiotic resistance of bacterial pathogens is one of the greatest worldwide dangers to general human services. To lessen choice and spread of resistance, the utilization of conventional anti-infection agents must be constrained and elective compelling treatments must be looked for. Bacteriocins get to be one of the weapons against microorganisms because of the particular attributes of extensive differences of structure and capacity and common asset. (MaruškaBudič,et al 2011)

Bacteriocins are extracellular substances

delivered by various sorts of microbes, including both Gram positive and Gram negative species. They can be delivered suddenly or affected by specific chemicals, for example, mitomycin C. They are naturally one of the essential substances, and have been observed to be helpful in layer contemplates furthermore in writing pathogenic microorganisms bringing about genuine nosocomial infections(Shih-Chun Yangat el 2014)

Bacteriocins are a heterogeneous compound of particles with various morphological and biochemical substances. They go from a basic protein to a high atomic weight complex:

the dynamic moiety of every particle in all cases is by all accounts protein in nature. The hereditary determinants of the greater part of the Bacteriocins are situated on the plasmids, aside from few which are chromosomally encoded. These bactericidal particles are species particular. They apply their deadly action through adsorption to particular receptors situated on the outside surface of delicate microorganisms, trailed by metabolic, natural and morphological changes bringing about the death of such microscopic organisms (Acuña, et al 2012)

Bacteriocins of Gram-positive microscopic organisms are as bounteous and significantly more various than those found in Gram-negative microorganisms. The Gram-positive bacteriocins take after a considerable lot of the antimicrobial peptides delivered by eukaryotes; they are for the most part cationic, amphiphilic, film permeabilizing peptides, and reach in size from 2 to 6 kDa (O. Gillor, et al 2008).

Gram negative bacteriocin delivering microbes are *E. coli*, *Salmonella enterica*, *Hafnia alvei*, *Citrobacter freundii*, *Klebsiella oxytoca*, *Klebsiellapneumoniae*, and *Enterobacter cloacae* uncover levels of bacteriocin creation running from 3 to 26% of natural separates. Colicins, bacteriocins created by *E. coli*, are found in 30–50% of the strains detached from human has and are regularly alluded to as harmfulness variables. Much larger amounts of bacteriocin creation have been found in some Gram-negative microorganisms, for example, *Pseudomonas aeruginosa*, in which >90% of both natural and clinical secludes produce Bacteriocins. (Daw et al 1996.)

Bacteriocins are accounted for to inhibit important animal and plant pathogens, for example, Shiga toxin delivering *E. coli* (STEC), enterotoxigenic *E. coli* (ETEC), methicillin-safe *Staphylococcus aureus* (MRSA), VRE, *Agrobacterium*, and *Brenneria* spp. The

bactericidal systems of Bacteriocins are essentially situated in the receptor-tying of microscopic organisms' surfaces, and after that through the film, which causes microbes cytotoxicity. Also, Bacteriocins are low-lethal peptides or proteins delicate to proteases, for example, trypsin and pepsin. (Shih-Chun Yang et al 2014, Acuña, et al., 2012)

In vagina bacteriocins generation by probiotic lactobacilli strains was found to hinder the development of some of these irresistible pathogens: *L. acidophilus* and *L. jensenii* strain 5L08 indicated adversarial movement against *G. vaginalis* likewise represses the development of *Candida albicans* (Shih-Chun Yang et al 2014)

Lactobacillus anticipates infections of the urogenital and intestinal tracts. The nearness and predominance of *Lactobacillus* in the vagina is connected with a diminished danger of bacterial vaginosis and urinary tract infections. (Roxanne Gavillon, et al., 2012)

Bacteriocin created by these *Lactobacillus* species had a vast range of hindrance against sustenance deterioration microorganisms and different related strains of lactic acid bacteria. The bacteriocin hindered the development of *Escherichia coli* however it demonstrated minimum action against *Candida albicans* (Felis, et al., (2007).

Lactobacillus Bacteriocins are found inside of each of the four noteworthy classes of antimicrobial proteins delivered by lactic acid bacteria. Class I Bacteriocins (anti-infection agents) were found in the *Lactobacillaceae*. These Bacteriocin small membrane-active peptides (<5 kDa) containing an unordinary amino acids, lanthionine. The class II bacteriocins are little heatstable, non-lanthionine containing and layer active peptides (<10kDa). The class III bacteriocins have been found in *Lactobacillus*, incorporate warmth labile proteins of vast atomic mass. The class IV Bacteriocins are a

gathering of complex proteins, connected with other lipid or starch moieties, which have all the earmarks of being required for action. They are moderately hydrophobic and heat stable (Parvathy *et al.* 2004)

Individuals from the family Bacillus are known a wide weapons store of antimicrobial substances, including peptide and lipopeptide anti-toxins, and Bacteriocins. A number of the Bacillus Bacteriocins fit in with the lantibiotics, a class of post-translational adjusted peptides generally spread among various bacterial clades. Lantibiotics are among the best-described antimicrobial peptides at the levels of peptide structure, hereditary determinants and biosynthesis mechanisms (Granum, *et al.* 2001).

Escherichia coli produce two sorts of bacteriocins, colicins and microcins (O. Gillor, *et al.*, 2008). Colicins have high sub-atomic mass, while microcins are ordinarily <10 kDa. Microcins can be either chromosomally or plasmid encoded, while colicins have been discovered just on plasmids (Marie-Pierre *et al.* 2014.). Colicin creation is generally corresponded with a SOS reaction to push (Frece J1 *et al.* 2005), and arrival of the colicin commonly happens through cell lysis. (Kang C *et al.*, 2004)

Microcins are discharged from in place cells (Pedersen, *et al.* 2002). Bacteriocins that execute contenders through pore arrangement or nuclease action or by hindering protein amalgamation have been distinguished (Hammami R *et al.* (2007)

Pyocins are created by more than 90% of *Pseudomonas aeruginosa* strains and every strain may incorporate a few pyocins. (Michel-Briand *et al.* 2002).

The pyocins genes are situated on the *P. aeruginosa* chromosome and their exercises are inducible by mutagenic specialists, for example, mitomycin C. Three sorts of pyocins

are depicted. (i). R-sort pyocins look like non-adaptable and contractile tails of bacteriophages. They incite a depolarization of the cytoplasmic layer in connection with pore arrangement. (ii). F-sort pyocins likewise look like phage tails, yet with an adaptable and non-contractile bar like structure. (iii). S-sort pyocins are colicin-like, protease-touchy proteins. (Michel-Briand *et al.* 2002)

Probiotics have advanced as a promising distinct option for antibiotics agents since they are known not assurance against pathogen attack by blocking attachment or poison receptor locales of human intestinal epithelial cells lessening gut pH, and creating antimicrobial compounds. A large portion of the acknowledged probiotic microscopic organisms fit in with the Gram-positive genera *Lactobacillus* or *Bifidobacterium* known not advantageous human gut commensals. One special case is the Gram-negative *Escherichia coli* strain, a nonpathogenic human confine that is asserted to rebalance the intestinal verdure after anti-microbial medications or other intestinal aggravations. This strain was accounted for to shield from infection with *Salmonella* serotype and its inhibitory limits were identified with the blockage of attachment locales in the human digestive system or to Bacteriocins creation. *E.coli* delivering microcin that represses the development of salmonella species. (A. Zihler1 *et al.* 2009)

MATERIAL AND METHOD

For isolation Bacillus from soil: Weight 1gm soil and inoculates it into 5ml nutrient broth, then incubates the tube at 37°C for 24 hours. After 24 hours transfer 0.1ml of supernatant of soil sample in nutrient agar plate and streak the plate with the help of wire loop then incubate the plate at 37°C for 24 hours.

For antibiotic susceptibility to penicillin: take a loopfull culture and inoculate it into saline for making suspension. Now take a loopfull

culture from suspension and inoculate or streak it on a Mueller Hilton Agar (MHA). Now from sterile forceps gently place an antibiotic disc of penicillin in the center of the streak plate. Finally incubate the plate at 37°C for 24 hours.

For isolation of *E. coli*: from sewage sample take a loopfull culture of sewage water sample and streak it on a plate of EMB agar. Then incubate the plate at 37°C for 24 hours.

For isolation *Pseudomonas*: 2 samples were taken: Take 1gm of soil and inoculate it into 5ml normal saline. From raw refrigerated milk. Take 0.1ml of raw refrigerated milk into normal saline. Then incubate the both the tubes at 37 degree centigrade for 24 hours. Next day take a loopfull culture from the tubes. Streak the plates of PS agar with both culture samples. Incubate the plates at 37°C for 24 hours.

For Isolation of *Lactobacillus* from Cheese: Dry Milk, Raw Milk and Yogurt 10gm of each sample were taken aseptically. Then each sample was homogenized in 90ml of sterile saline by using homogenizer. Make fivefold dilutions of the homogenates were prepared. Then inoculated the loop full sample of each homogenates on plates of MRS agar plate. Finally incubate the plates an aerobically at 32°C for 48 hours.

For Bacteriocins Production from isolated culture: Inoculate the confirmed culture of Bacillus, *E. coli*, *Pseudomonas* into nutrient broth and *Lactobacillus* into MRS broth; leave the tubes for 20 days at room temperature. After incubation, cells were removed from the growth medium by centrifugation (10,000×g for 15 min, 4°C). The resultant supernatants are bacteriocins and can be stored at -20°C.

For Estimation of Bacteriocins Production: A standard curve was prepared by taking BSA as a standard protein. The standard curve was prepared by taking the following amounts of water, BSA and Bradford reagent: Standard

curve for protein estimation Standard. Now after we have prepared a standard curve we check the OD of all the samples by putting them in volume. 3ml Bradford reagent + 50µl sample + 150µl water. The O.D of supernatants were observed.

For Agar Well Diffusion Method: The strains of pathogenic organism used to study the antimicrobial activity. MHA is used to test the antimicrobial activity against these pathogens. 0.1ml dilutions of each pathogen were tested by pour plate method. Four wells were made by using sterile borer. Then add pure cultures (20 days inoculated samples) were added in the wells. Results were recorded for 3 days incubation with 24 hours interval.

For Agar over Lay Method: The strains of pathogenic organism used to study the antimicrobial activity. MHA is used to test the antimicrobial activity against these pathogens. 0.1ml dilutions of each pathogen were tested by pour plate method. Plates were incubated anaerobically for 4 days for growth. Then after 4 days another layer of media having either pure culture or isolated cultures was over laid over it. Results were recorded after 4 days anaerobic incubation.

RESULTS

Morphological Characteristics of Microbial Isolates: Bacillus is a large disc like; flat, irregular, opaque, creamish of white colonies appears on nutrient agar plate of bacillus. In microscopy gram positive purple colour, Spores are also seen. Some spores are central and some are present at terminal position. Catalase negative No clear zone appear on the plate of MHA indicates the resistivity of bacillus against penicillin.

***E. coli*:** Small smooth opaque colonies appear on EMB plate of *E. coli*. Gram negative rods seen in the microscopy, Catalase test positive. TSI tubes turn yellow after incubation indicates the acid production.

Pseudomonas: Large, irregular, opaque, yellowish green colonies are appears in soil sample plate of Pseudomonas .No colonies appears on milk sample plate. Catalase positive (Bubbles formation), Blue colour appear on filter paper indicates the positive result of oxidase test.

Lactobacillus: Small and large smooth creamish off white colonies on MRS agar plate of lactobacillus. In raw milk or yogurt purple gram positive rods are seen. In cheese or dry milk, gram positive rods seen along with gram negative rods. All samples are catalase negative

Bacteriocin Production From Isolated Culture: Spectrophotometer at 520 nm, optical densities of isolated organisms are shown in tables no 1. The antagonism was observed against pathogenic organism. Zone of inhibition are observed. Bacteriocins from lactobacillus(milk) show largest zones. In agar over lay method: Clear patches indicate the anti-adhesive

property of Lactobacillus, Pseudomonas, E.coli, and Bacillus against test pathogens. Rings indicate biofilm formation. Bacteriocins from isolated culture show zones of inhibition in agar well diffusion method against pathogenic organisms. In agar over lay method clear patches represents the antagonistic effects of Bacteriocins. In figure no 1 the percentage of OD is shown. In figure no 2 percentage of bacteriocins is shown. In table no 2 the results of agar well diffusion is described.

Table I: OD of the organisms

ORGANISM	O.D
E.coli P	0.223
E.coli H	0.205
Pseudomonas Hos	0.208
Bacillus	0.155
Lactobacillus Milk	0.196
Lactobacillus Yogurt	0.196

Table II: Zone of inhibition of bacteriocin against m. organisms

WELL DIFFUSION METHOD					
BACTERIOCIN	PATHOGENIC ORGANISMS				
	<i>E.coli</i>	<i>Aspergillus</i>	<i>P.aeruginosa</i>	<i>S.aureus</i>	<i>S. typhi</i>
Lactobacillus milk	36mm	15mm	38mm	20mm	28mm
Lactobacillus yogurt	38mm	20mm	35mm	22mm	25mm
E.coli	–	0mm	25mm	0mm	0mm
Pseudomonas	20mm	11mm	–	17mm	28mm
Bacillus	–	–	27mm	–	–

DISCUSSION

Bacteriocins are naturally one of the imperative substances, and have been observed to be helpful in layer concentrates on furthermore in writing pathogenic microorganisms bringing on genuine nosocomial diseases. Bacteriocins are a heterogeneous group of particles with various morphological and biochemical elements. They extend from a basic protein to a high sub-atomic weight complex: the dynamic moiety

of every particle in all cases is by all accounts protein in nature. The hereditary determinants of a large portion of the bacteriocins are situated on the plasmids, aside from few which are chromosomally encoded. These bactericidal particles are species particular (O. Gillor *et al.*, 2008)

All in all, the gene and immune gene of bacteriocins are encoded on the same plasmid or in neighboring districts of a chromosome. The bacteriocins qualities can go into other

bacterial cells by means of conjugation (Shih-Chun *et al.*, 2014).

In our study we detach the bacteriocin delivering life forms from which we create bacteriocin which has the bacteriostatic or bactericidal impact on pathogenic living beings. This adversarial movement of bacteriocin is used as a force full weapon in pharmaceuticals items which battle against the pathogens. Nisin and different bacteriocins created by lactic acid bacteria have gotten a lot of consideration since they are delivered by microorganisms to a great extent considered helpful to human wellbeing and to sustenance generation. (R. D. Joerger *et al.*, 1971)

Every one of the strains of lactobacillus, E.coli, Pseudomonas and Bacillus created the proteinaceous compound bacteriocin which has a high antimicrobial movement against all tried pathogenic microbial strains and goes about as expansive range antimicrobials. In this study the most elevated antimicrobial movement of bacteriocin delivered from Lactobacillus microorganisms (crude milk & yogurt test). The most noteworthy zone of restraint was seen against Gram-negative bacteria E.coli Pseudomonas, S.typhi. Gram-positive organisms' *Staphylococcus aureus* likewise have bactericidal movement against bacteriocin. fungus *Aspergillus* additionally indicate middle of the road zone of restraint.

CONCLUSION

It is concluded that Lactobacillus, E.coli, Bacillus and Pseudomonas produced bacteriostatic or bactericidal compound that have antagonistic effect against pathogenic organisms. So we can use bacteriocins as a therapeutic tool in medical pharmaceuticals to fight against pathogenic organisms.

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