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ISOLATION AND IDENTIFICATION OF BACITRACIN PRODUCING BACILLUS SPECIES FROM DIFFERENT MILK SAMPLES

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ABSTRACT

The present study involves the isolation and identification of bacitracin producing *Bacillus* species from different milk samples. The genus *Bacillus* produces mainly polypeptide antibiotics such as bacitracin and polymyxin. Bacitracin is a constituent in Neosporin ointment for controlling skin infections. It was isolated by spread plate technique. It was inoculated into the fermentation medium. All were screened for the production of antibiotic by agar diffusion assay against *Staphylococcus aureus*. Antibacterial activity was measured in terms of zone of inhibition (mm). The *Bacillus* isolate B8 showing maximum zone of inhibition. Since bacitracin is effective against gram positive organism, the obtained antibiotic was identified as bacitracin by cup plate method using *E.coli* as test organism. The high rate of resistance of isolated *Bacillus* species against bacitracin, confirmed it as bacitracin producer.

KEYWORD

Bacillus species, Bacitracin and Antimicrobial activity.

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INTRODUCTION

The word “antibiotic” is derived from Greek term antibiosis, which literally means “Against life”. It can be purified from microbial fermentation and modified chemically or enzymatically for basic research^{1, 2}. More than 5000 different antibiotics have been isolated from cultures of bacteria, fungi and plant cells, 60% of them are contributed by the genus *Streptomyces*^{3, 4}. *Bacilli* are rod shaped gram positive, sporulating aerobes or facultative anaerobes. Most *Bacilli* are saprophytes. Each

bacteria creates only one spore, which is resistant to Bacitracin is a mixture of several polypeptides differing in their amino acid composition⁶. It was first discovered in 1943 and named after a culture of bacillus and the last name of a seven year old American girl Margaret Tracy, from whose wounds the Bacillus was isolated⁷. It is an ingredient in several commercially available topical triple antibiotic ointment such polysporin and Neosporin that are used to prevent infection in minor cuts and burns.

Milk is the major, repository of microorganisms that produce antibiotic capable of inhibiting the growth of other microorganisms. Clinically useful antibiotics have been isolated from four groups of milk microorganisms *Streptomyces*, *Bacillus*, *Penicillium* and *Cephalosporium*. Although milk from all parts of the world are continually screened in industrial laboratories for the isolation of new antibiotic producing microorganisms, industrial microbiology is directing its energies toward chemical modification of existing antibiotic substances.

MATERIAL AND METHODS

Isolation of *Bacillus* species

Milk samples were collected from different sources such as cow, buffalo and goat. 10 ml sample in each test tube were given heat shock at 75^oC for 15 minutes by placing in water bath. Then, they were cooled abruptly to 10^oC by placing in chilled water. 0.5ml of above samples were poured into petri plates with tryptone yeast extract medium containing 5g of tryptone, 2.5g of yeast extract, 1.0g of glucose, 20g of agar and 5IU/ml bacitracin. The petri plates were incubated at 37^oC for 24 hours. Isolated colonies were then picked up and subcultured on TYE (tryptone yeast extract) agar slants and incubated for 24 hours at 37^oC. The pure culture of *Bacillus* species were labeled and stored in refrigerator.

Characterization of isolated strains

The isolated strains were studied for morphological, cultural and biochemical characteristics. For screening, the vegetative cultures thus obtained were

heat, cold, radiation, desiccation, disinfectants⁵.

Used for inoculation into fermentation medium. The following tests were carried for the preliminary identification of milk isolates by simple staining, gram staining, and motility test. The gelatin hydrolysis test, starch hydrolysis test, Indole production test, methyl red test were carried out for the milk isolates.

Bacitracin production by *Bacillus* species

Inoculum preparations

A loopful bacterial growth of isolates of *Bacillus* species from 24 hours old TYE agar slants were aseptically transferred to separate flasks with 25ml sterile inoculum medium containing 10g of peptone, 5.0g of glucose, 5.0g of beef extract, 2.5g of sodium chloride, 0.167g of MnCl₂ in 1 liter of distilled water (pH 7) and flask were incubated at 37^oC in an shake flask for 24 hours.

Production medium

4% v/v inoculum was used to inoculate 50ml sterile fermentation medium. The inoculated flasks were incubated at 37^oC in shake flask for 48 hour. The culture was centrifuged.

Agar well diffusion method

It was used to check level of antibiotic in culture filtrate using *Staphylococcus aureus* as test organism and nutrient agar plates. 24 hours fresh culture of *staphylococcus aureus* was diluted with presterilized normal saline and the turbidity of culture was adjusted to 0.5 McFarland with presterilized normal saline. Bacterial lawn (using 0.2ml test culture) and wells (6mm in diameter) were prepared in nutrient agar plates. 100 micro liter of cell free supernatants were pipette into the wells. The agar plates were incubated at 37^oC for 24 hours. After 24 hours, the zone of inhibition were observed and measured.

Identification of Bacitracin Producer

Since bacitracin is effective against only gram positive organism, the obtained antibiotic was identified as bacitracin by cup plate method using *E.coli* (Gram negative) as test organism. The high rate of resistance of isolated *Bacillus* species against bacitracin confirmed it as bacitracin producer.

RESULTS AND DISCUSSIONS

Isolation of *Bacillus* species

Several pure cultures of bacillus species were isolated from buffalo, goat and cow milk samples. The result are shown in Figure No.1 is isolated species from buffalo milk, Figure No.2 isolated species from cow milk and Figure No.3 isolated species from goat milk.

The results indicated that, the presence of bacillus species are less in cow (Figure No.2) and goat (Figure No.3) of milk than the buffalo (Figure No.1).

Characterization of isolated strains

Preliminary identification of isolates

The preliminary identification of milk isolates was performed and the results indicated that the morphological characteristics of strains B1 to B8 were bacilli. The gram staining techniques showed that all strains were Gram Positive and from the motility test all strains were found to be active motile (Table No.1).

Biochemical reactions of milk isolates

The biochemical tests results revealed that all the strains had starch hydrolyzing property. Gelatin hydrolysis test, Indole production test, methyl red test were negative for all strains. This showed that all isolated strains were bacillus species (Table No.2).

Bacitracin production by *Bacillus* species

After 48 h of fermentation 0.1 ml of filtrated fermentation media was dropped in each well in nutrient agar swab with *Staphylococcus aureus*.

Zone of inhibition (Figure No.4) indicates the presence of antibiotic in fermented medium.

A total of 8 bacterial strains were found to be producing zone of inhibition out of which one species was selected on the basis of maximum zone of inhibition for further studies. Table No.3 shows antibiotic potential of isolates. Fermented broth of B8 isolate of *bacillus* species showed maximum zone of inhibition (13mm). Zone of inhibition in Graph (Figure No.5).

Identification of Bacitracin Producer

Bacitracin is an antibiotic which is found to be active against Gram positive bacteria. Zone of inhibition found around the cavity containing Ciprofloxacin and Gentamycin. However, no zone of inhibition was found around the cavity with fermented liquid and standard (Figure No.6). Since, the fermented liquid does not inhibit Gram negative bacteria; the fermented liquid may contain bacitracin.

Confirmation of identified bacitracin producer as bacitracin resistant

Most of the microorganisms that produce antibiotics are resistant to the action of their own antibiotic, although the organisms are affected by other antibiotics and their antibiotic may be effective against closely-related strains. No zone of inhibition around the cavity with fermented liquid, Zone of inhibition around cavities with penicillin, ciprofloxacin (Figure No.7). This indicated that, the isolated identified organism was bacitracin resistant. This confirmed the isolated *Bacillus* species is a bacitracin producer.

Table No.1: Preliminary identification of isolates

S.No	Type of Identification	B1	B2	B3	B4	B5	B6	B7	B8
1	Morphology	Bacilli	Bacilli	Bacilli	Bacilli	Bacilli	Bacilli	Bacilli	Bacilli
2	Gram Staining	+ve rod	+ve rod	+ve rod	+ve rod	+ve rod	+ve rod	+ve rod	+ve rod
3	Motility Test	Active Motile	Active Motile	Active Motile	Active motile	Active motile	Active motile	Active motile	Active motile

Table No.2: Biochemical reactions of milk isolates

S.No	Type of Identification	B1	B2	B3	B4	B5	B6	B7	B8
1	Gelatin Hydrolysis Test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
2	Starch Hydrolysis Test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
3	Indole Production Test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
4	Methyl Red Test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

Table No.3: Antibacterial activity of isolates of *Bacillus* species

S.No	<i>Bacillus</i> species (Isolate no.)	Antibacterial activity (zone of inhibition in mm)	<i>Bacillus</i> species (Isolate no.)	Antibacterial activity (zone of inhibition in mm)
1	B1	5	B5	7
2	B2	9	B6	11
3	B3	2	B7	10
4	B4	12	B8	13



Figure No.1: Isolated species from buffalo milk

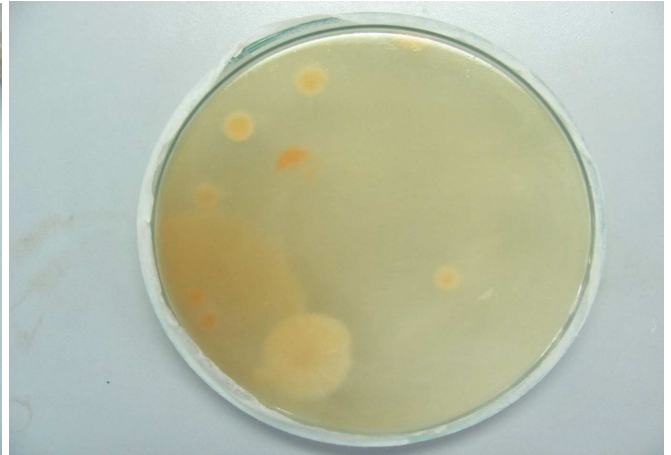


Figure No.2: Isolated species from cow milk



Figure No.3: Isolated species from goat milk



Figure No.4: Presence of antibiotic in fermented medium

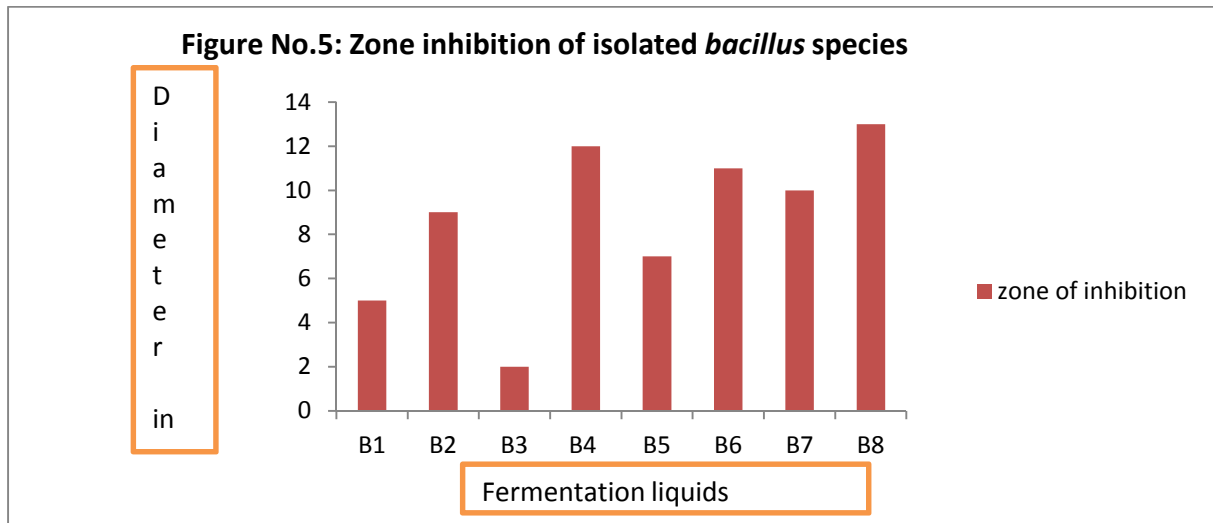


Figure No.5: Zone inhibition of isolated bacillus species



Figure No.6: Identification of Bacitracin



Figure No.7: Confirmation of identified bacitracin producer as bacitracin resistant

CONCLUSION

The isolated bacillus species were present in various sources of milk; it was found that efficient bacitracin producing bacilli were present more in

buffalo milk than cow and goat. The isolates B1 to B8 was found to be Bacilli and were active motile. All isolates were found to be gram positive. Our biochemical study results indicated that all the

isolates exhibited positive results for the sources of starch hydrolysing enzyme. Antibacterial activity of the fermented liquid showed a clear inhibition zone in the media seeded with *Staphylococcus aureus*. Since, Bacitracin is effective against Gram positive organism, the resistance of E.coli to fermented liquid indicates the presence of bacitracin in the medium. In conclusion, the study was achieved the isolation of Bacillus species which produce antibiotic, bacitracin.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

REFERENCES

1. Satpal S B *et al.* Isolation, purification and characterization of bacitracin from *Bacillus* species, *International journal of pharmacy and pharmaceutical sciences*, 3(4), 2011, 136-138..
2. De Mondena J A, Guttierrez S A J, Falchini R A, Gallazo J L, Hughes D E, Bailey J E and Martin J F. Intracellular expression of vitreoscilla haemoglobin improves Cephalosporin C production by *Acremonium chrysogenum*, *Biotech*, 11(8), 1993, 926-929.
3. Todar K. Antimicrobial Agents used in the treatment of infectious disease, *Department of Bacteriology University of Wisconsin-Madison*, 55, 2002, 619-26.
4. Claus G W and Blackwell. Antibiotic Evaluation by Kirby Bauer Method. In: *Understanding Microbes, a Laboratory Textbook for Microbiology*, Amazon, First Edition, 1st edition, 1989, 405.
5. Muhammad Awais *et al.* Isolation, Identification and optimization of bacitracin produced by *bacillus* species, *pak j Bot*, 39(4), 2007, 1303-1312.
6. Hammes E J and frank, M P. Bacitracin Chemistry, Metabolism, dietary sources, *Proc.Nat.Acad.Sci*, 17(2), 1979, 405-417.
7. Meleney F L. Structural role of the cell surface glycoprotein of *Halobacterium salinarium*, *J.Biochem.pharmacol*, 17(2), 1946, 137-145.
8. Arky R. Bacitracin is a metal dependant peptide antibiotic from cultures of *Bacillus*, *J. of Serbian society*, 69(11), 1997, 883-886.
9. Dyck K H, and Samsons T. Allergic effects on using antibiotics, *J. on medical science*, 54(3), 1997, 540-561.

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