

Antagonistic activity of Bacitracin on *Staphylococcus aureus*

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Abstract: Bacitracin (C₆₆H₁₀₃N₁₇O₁₆S) is a metal dependent complex mixture of cyclic polypeptides that is produced by *Bacillus licheniformis* and *Bacillus subtilis*. Bacitracin consists of mixture of structurally similar polypeptides from 12 amino acids. Bacitracin has wide spread application in pharmaceutical for the treatment of chronic sinusitis, *Clostridium difficile* associated disease, necrotic enteritis, topical antibiotic feed additive and reduction of diseases in poultry. Bacitracin is produced by many bacteria but well known organism for the production of bacitracin is *Bacillus subtilis*, also known as the hay *Bacillus*, it is a gram+ve, catalase positive bacterium commonly found in soil. *B.subtilis* is a member of the genus *Bacillus*, is rod shaped and has the ability to form through, protective endospore. The ability of the antibiotics to suppress protein synthesis was determined by exposing *cocci* to an inducing substrate in the presence of antibiotic and observing the extent of inhibition of induced enzyme formation. Isolation and screening of the isolates was carried out on the basis of bacitracin production (IU/ml) and was determined by agar diffusion method. In this study both physical (UV for 15 and 20minutes) and chemical (MNNG and HNO₂ for 15 and 20minutes) mutagens were employed in systematic manner to obtain mutants that gave higher bacitracin production in the treatment of UV for 20minutes and 15minutes in chemical mutagens. Minimum inhibitory concentration method was used to check the cultures for the production of antimicrobial metabolites against *Staphylococcus aureus*. The results indicated that, the test organism, *S. aureus* was inhibited to the extent of 0.8 to 1.9cm in normal production method and 0.7 to 1.4cm in chemical method by end of the study *i.e* seventh day after initiation of the study. Whereas, the inhibition of *S.aureus* was not significant in physical method.

Key words: *Bacillus subtilis*, *Staphylococcus*, Bacitracin antibiotic, minimum inhibitory concentration method

INTRODUCTION

Bacitracin is a narrow spectrum antibiotic directed primarily against gram-positive bacteria such as *Staphylococcus* and *Streptococcus* via inhibition of cell wall synthesis but also has a little activity against gram-negative organisms. It is most commonly used in complex with zinc which seems to stabilize the antibiotic complex. It is poorly absorbed from the gastrointestinal tract as well as from skin and mucosal surfaces. Absorbed bacitracin is excreted by glomerular filtration (Prescott and Baggot, 1993).

Production and utilization of bacitracin plays a key role in bio-processing and has wide spread applications. Bacitracin is being used in treatment of many human diseases like bacterial endocarditis, osteomyelitis, bacteremia, meningitis and intracranial infections, surgical infections, infections of eye and ear, chronic sinusitis, *Clostridium difficile* associated disease, gastrointestinal infections, necrotic enteritis and topical treatment). Bacitracin is also being used in poultry and veterinary as growth promoter (Sims *et al.*, 2004; Thibodeau *et al.*, 2008) and as veterinary medicine for the treatment of different diseases in piglets, poultry and turkey and quails. The branched cyclic dodecylpeptide antibiotic bacitracin produced by several strains of

Bacillus licheniformis and *Bacillus subtilis* (Azevedo *et al.*, 1993;) is synthesized non-ribosomally by the large multienzyme complex bacABC (Konz *et al.*, 1996). Non-ribosomally synthesized peptides can be described as peptides elaborated in bacteria, fungi and streptomycetes that contain two or more moieties derived from amino acids (Kleinkauf and von Dohren, 1988).

Bacitracin has a molecular weight of about 1470 and a possible empirical formula of $C_{65}H_{103}N_{16}O_{17}S$. Acid hydrolysis of bacitracin A yields a mixture of amino acids including L-leucine, L-isoleucine, L-cystein, L-histidine, L-lysine, D-phenylalanine, Dornithine, D-glutamic acid and DL-aspartic acid. By partial hydrolysis of bacitracin A, peptide fragments have been isolated which gave an indication as to the probable arrangement of amino acids in the intact molecule The induced mutation followed by strain selection is a routine practice for improving many bacterial and fungal metabolites that primarily includes antibiotics and enzymes.

Antibiotics are low molecular weight (non protein) molecules produced as secondary metabolites, mainly by micro organisms that live in the soil. A natural assumption is that soil microbes produce antibiotics in their natural habitat

Bacitracin is a branched cyclidodecyl peptide produced by *Bacillus licheniformis* and some strains of

Bacillus subtilis species. Bacitracin chemical formula is $C_{66}H_{103}N_{17}O_{16}S$. Bacitracin consists of a mixture of structurally. It has also been reported that bacitracin has no negative impact on human health.

Production of bacitracin can be observed by treating the bacillus species with physical and chemical mutagens. Vegetative cells of *Bacillus subtilis* were mutated with UV irradiation & ethidium bromide. The bacillus species play main role in production bacitracin production. *Bacillus subtilis*, known also as the hay bacillus, is a gram –positive, catalase –positive bacterium commonly found in soil. A member of the Genus bacillus, *B.subtilis* is rod shaped and have the ability to form a tough ,protective endospore.

Methods and materials

Production of bacitracin:

Inoculum preparation(Normal production):

Inoculum was prepared by inoculating the bacitracin producing bacillus species in to the nutrient broth and incubating at 37°C for 72 hours in an orbital shaker at 120 rpm

Mutant production:

Physical mutation: The inoculum was treated with U V irradiation for 20 minutes.

Chemical mutation: The inoculum was treated with 50µ litres of Ethidium bromide and kept for production of antibiotic.

Production medium:

About 10% inoculum (normal, physical& chemical) of isolate was added in separate flasks containing 100 ml synthetic media for checking the bacitracin production the flasks were incubated for 30°C in an orbital shaker at 150 rpm after every 24 hours samples were taken up to 144 hours, centrifuged at 10000 rpm to get cell free supernatant s, which were sterilized through 0.2µm filter paper.

Composition of synthetic media:(g/l)PH-7

L-Glutamic acid	-	5.0
KH ₂ PO ₄	-	0.5
K ₂ HPO ₄	-	0.5
MgSO ₄ .7H ₂ O	-	0.2

MnSO ₄ .7H ₂ O	- 0.01
Nacl	- 0.01
FeSO ₄ 7H ₂ O	- 0.01
CuSO ₄ .7H ₂ O	- 0.01
Cacl ₂ .2H ₂ O	- 0.015
Glucose	- 10

Minimum Inhibition Concentration Method:

This MIC method was used to check the cultures for the production of antimicrobial metabolites (Sen *et al.*,1995).Twenty four hours fresh cultures of *Staphylococcus aureus* was spread on surface of the nutrient agar plates and then wells were prepared over the nutrient agar plates. About 80µlit cell free supernatants were added in the wells and the plates were incubated at 37° C for 24 hours, the zone of inhibition were observed and measured.

Optimization of cultural parameters:

Incubation period (0 to 20 hours), initial pH of the production medium (6 to 9) and glucose concentration (1 to 5%) was optimized for maximum production of antibiotics by immobilized whole cells of *B.subtilis*.

B.subtilis were incubated at 30° C in an orbital shaker at 150 rpm and sample was drawn at 0 hour and then after every 4 hours, from 4 hours to 20 hours in case of optimization , and at 0 hour and after 4 hour of incubation for pH and glucose optimization.

Optimization of cultural parameters:

Effect of incubation period:

Normal production: Maximum production of antibiotic was obtained after 24 hours of incubation period. Although significantly good amount was also found at 3-5 days of incubation period

Physical mutant production: Maximum production of antibiotic was not observed in case of physical mutated organism.

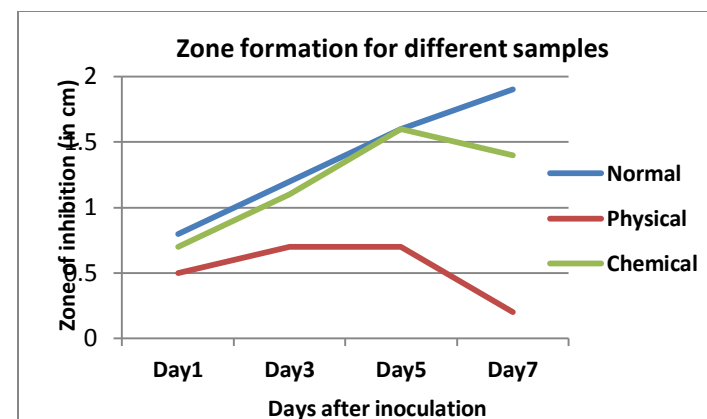
Chemically mutated production: Maximum production of antibiotic can be observed after 24 hours incubation period. From the second day the antibiotic activity was decreased.

Normal

Physical mutant



Chemical mutant



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CONCLUSION:

The *Bacillus subtilis* organisms can be isolated from soil by using soil sprinkle technique. Morphological and biochemical techniques were performed to identify the characterization of *Bacillus subtilis*. Inoculums were prepared in nutrient broth by inoculating *Bacitracin* producing organism. For the screening of *Bacitracin* was done in specific synthetic medium and incubated at 30°C for 7 days in an orbital shaker at 150 rpm.

MIC method for antibiotic activity:

Production	Day1	Day3	Day5	Day7
Normal	0.8	1.2	1.6	1.9
Physical	0.5	0.7	0.7	0.2
Chemical	0.7	1.1	1.6	1.4

Zone of inhibition measurements in cm

Both Physical and Chemical mutants were also prepared and incubated 7 days at 30°C in an orbital shaker. After filtration was over the filtrate was used to perform MIC (Minimum inhibitory concentration) and Identification of peptide antibiotic by Paper Chromatography methods.

Normal production of *Bacitracin* shows Maximum inhibition. Physical and Chemical mutant productions shows less zone formation. "Normal production and Chemical mutant antibiotic productions shows action on *Staphylococcus species*".

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