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INDUCED ALTERATIONS OF BACTERIOCIN FROM *"PSEUDOMONAS AERUGINOSA"*

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ABSTRACT

The term ''bacteriocin'' was coined by Jacob in 1953. These molecules are defined as ribosomally synthesized anti-microbial peptides, proteins or proteinaceous complexes produced by bacteria that act mainly against closely related species. They were originally defined as proteins characterized by lethal biosynthesis, predominantly intra-species killing activity and adsorption to specific receptors on the surface of bacteriocins sensitive cells. Bacteriocin production is widespread among bacteria. It has been suggested that the majority of bacterial species synthesize bacteriocins. The mechanism of inhibition of pathogenic bacteria for several of those probiotic microorganisms is mediated by the production of bacteriocins. The application of bacteriocins such as colicins in livestock has been largely achieved by feeding bacteriocin-producing strains.

KEYWORDS: Pseudomonas aeruginosa, bacteriocin, microcins and colicins, Gram negative and positive bacteria.

INTRODUCTION

Bacteriocins are prokaryotic antimicrobial proteins or antimicrobial peptides. As many of these substances reported in the literature have a broad spectrum of action (Heng *et al.*, 2007), exhibiting antagonistic activity against several pathogens, they have potential biotechnological applications (Bastos and Ceotto, 2011, Cotter *et al*, 2013). Due to the low availability of new drugs that could be used to control drug-resistant pathogens, bacteriocins have become an important option. Gram-positive bacteria are the major source of bacteriocins examined for biotechnological applications. Historically the term "Bacteriocin" was applied to antibiotic like compounds with specificity primarily restricted to bacterial strains. This can be thought of as microbial "murder" of one's relative, but their specificity

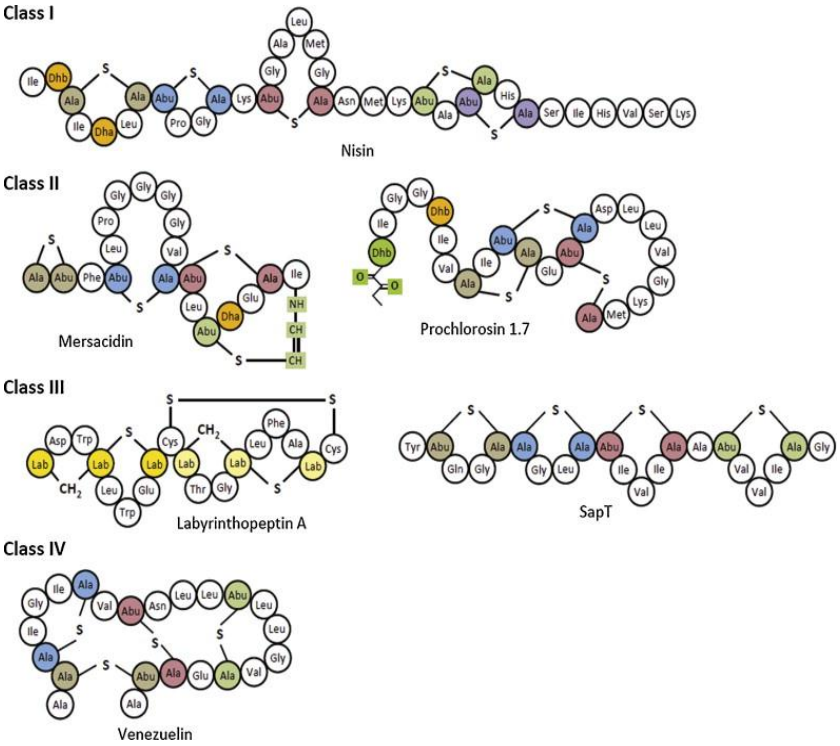
and chemical composition served to distinguish them from so called classical antibiotics. More than 99% of bacteria can produce at least one bacteriocin, most of which are not identified. The killing ability of bacteriocins is considered a successful strategy for maintaining population and reducing the numbers of competitors to obtain more nutrients and living space in environments. Moreover, the use of antibiotics or residues in food is illegal. Unlike chemical preservatives and antibiotics, "generally recognized as safe" (GRAS) bacteriocins, such as nisin, promise safe use as a food preservative in vegetables, dairy, cheese, meats, and other food products, as they inhibit microorganisms contamination during the production process (Deegan *et al.*, 2006, Settanni and Corsetti, 2008).

Bacteriocin production is widespread among bacteria. It has been suggested that the majority of bacterial species synthesize bacteriocins (Riley and Wertz, 2002). This is because their biosynthetic machineries are relatively simple and are often associated with transferable elements such as conjugative transposons or plasmids. As highlighted earlier, bacteriocins are ribosomally synthesized peptides. Generally related to bacteriocin biosynthesis are generally clustered, and are encoded on plasmids, chromosome or transposons with minimum genetic machinery consisting of structural cognate immunity genes. Bacteriocins are usually synthesized as biologically inactive prepeptides that include an N-terminal leader peptide attached to the C-terminal propeptide (Chen and Hoover, 2003). The bacteriocins have been grouped into different classes based on the different criteria such as producer organisms, molecular sizes, physical properties, chemical structures, mode of actions (Nes *et al.*, 2007) which sometimes resulted in different names for the same compounds (e.g. thiolbiotics and lantibiotics; microcins and colicins, bacteriocins). Bacteriocins are seen in both Gram-negative and Gram-positive bacteria. Bacteriocins from Gram-positive bacteria have attracted much interest for many reasons: they are frequently found in many commercially useful lactic acid bacteria LAB (e.g. *lactococci, lactobacilli, pediococci*) and those bacteria are generally regarded as safe (GRAS) for human consumption, since they are found or used in food and feed fermented products (FAO/WHO, 2002).

Pseudomonas aeruginosa is a gram-negative, rod shaped bacteria that belongs to the family *Pseudomonadaceae*. These pathogens are widespread in nature, inhabiting soil, water, plants and animals (including humans). It is an opportunistic pathogen and rarely cause disease in healthy persons, but can multiply easily in immune compromised patients (Balasubramanian *et al.*, 2013). The bacterium is responsible for severe nosocomial infections. According to the

survey conducted by the US National Healthcare Safety (2007), out of approximately 28,000 cases of nosocomial infections from 463 hospitals over a 22 month period, *P. aeruginosa* was found to be the sixth most repeatedly occurring pathogen, the second most frequent cause of ventilator-associated pneumonia and the seventh commonest cause of catheter-related bloodstream infection. Patients with burn wounds, AIDS and cystic fibrosis (CF) are at high risk of developing serious Pseudomonas infection which accounts for high death rate in this population (Valderrey *et al.*, 2010).

CLASSES-BACTERIOCIN



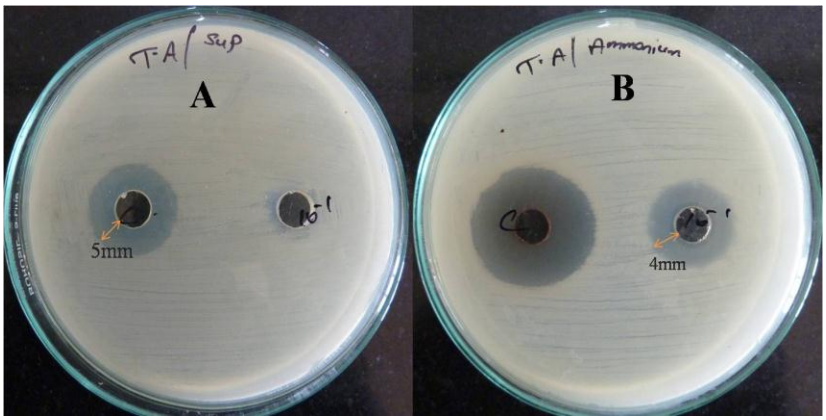
METHODOLOGY

Detection of antibacterial strains

The antibacterial activity of the isolates was determined by a deferred-antagonism plating assay (Tagg *et al.*, 1976).

RESULTS

Ammonium sulphate precipitation at 0-70% saturation was carried out. The maximum antibacterial activity was retained with 70% fraction. The cell free supernatant has lower (50AU/ml) effect on the growth of bacteria. The cell free supernatant after Ammonium sulphate precipitation exhibited high (400AU/ml) bactericidal activity was observed.



Antimicrobial activity of bacteriocins: A. Cell free supernatant: 50AU/ml; Ammonium sulphate precipitation: 400AU/ml

Table 1: Effect of chemicals and enzymes on bacteriocin.

Chemicals (Concentration 100µl)	Treatment (2hours) Incubation Zone (mm)	Treatment(4hours) Incubation Zone (mm)
SDS (1%)	4	4
NaCl (1%)	2	2
EDTA (1%)	5	6
Urea (1%)	2	4
TCA (1%)	3	4
Tween-80 (1%)	4	4
TritonX100 (1%)	5	5
Trypsin (1%)	3	3
α – amylase (1%)	2	3

DISCUSSION

Bacteriocins are initially isolated from Gram-negative bacteria. A colicin from *E. coli*, identified as an antimicrobial protein was the first described one of the bacteriocin family. In this discovery the colicins have been the most extensively studied Gram negative bacteriocins and they now serve as a model system for investigating the mechanisms of bacteriocin structure/function, genetic organization, ecology and evolution. A variety of probiotic bacteria have been tested to control animal and food borne pathogenic bacteria in livestock, but in many of them the beneficial effects have not been fully elucidated. The mechanism of inhibition of pathogenic bacteria for several of those probiotic microorganisms is mediated by the production of bacteriocins. The application of bacteriocins such as colicins in livestock has been largely achieved by feeding bacteriocin-producing strains, but there is very little evidence that administering bacteriocins alone to livestock (Mill, *et al*., 2013).

In recent years, the increased number of multi-drug resistant pathogens has become a serious problem, and finding or developing a new generation of antimicrobial agents is becoming increasingly important. Alternate methods for controlling pathogenic bacteria by the production of antimicrobial peptides called bacteriocins are now highly considered. Bacteriocin production is a widespread phenomenon among prokaryotes. Bacteriocins from Gram positive organisms, such as lactic acid bacteria have attracted much attention and have been the subject of intensive investigation due to their ability to act as a bio preservative agent in dairy foods and also in human therapeutic applications as antimicrobial agent. The number of Methicillin resistant *S. aureus* (MRSA) infection has been increasing and becoming a serious problem in public health worldwide. Novel antimicrobial agents are immediately needed to combat this drug resistant problem. The use of bacteriocin as an alternative agent to overcome the problem is promising (Papagianni, 2003). Hence in this study, bacteriocin producing bacteria was characterized. It is well documented with *P. aeruginosa* is produced bacteriocin was previously reported (Sehar Afshan Naz *et al.*, 2015).

The sensitivity of isolated *P. aeruginosa* was heat stable activity at 40°C. The effect of temperature on the stability of bacteriocin revealed that the compounds was relatively stable up to 40ºC for 10 minutes. Further heat treatment led to loss of activity. Further this bacteriocin activity was carried out at different time ranging from 10 to 100 mins. Maximum bacteriocin stability found to be at 70 mins. In addition, it was stable within a wide range of pH 7. High thermo stability is in contracted to previously report where R – type pyocin lost activity at a temperature above 40°C. Regarding the influence of pH on the biological activity, pyocin SA189 remained stable within the pH range 2 to 11. Retention of bioactivity of the pyocins at various pH values has been reported earlier (Saleem *et al.*, 2009). The present results shows that increasing time duration of bacteriocin treatment (3h, 6h) produced increasing dead cells of *S. aureus*. (A) Control 43.9% dead cells, (B) 3h treatment 60.5% dead cells, (C) 6h treatment 66.5% dead cells. The bacteriocin activity dead cell was increased in different time duration compared to control, within the area of bacteriocin research, flow cytometry has potential as a powerful tool for the investigation of cell membrane damage and repair mechanisms. However, suspensions of cells previously attached to solid surfaces have been analyzed by flow cytometry.

The FT-IR spectrum of *Pseudomonas aeruginosa* TA6 bacteriocin treated *S. aureus*. The strain was revealed that functional range at 300 and 4000 cm^{-3}.

In bacteriocin treated cells shift in absorbance in low frequency at 671.70, 1637.27, 2083.07 and 3460.46 cm^{-1}. FT-IR spectroscopy has been applied as a reliable method to study the putative mode of action of cell lytic bacteriocins from *P. aeruginosa* on *S. aureus* (Motta *et al.*, 2008). The main changes observed under SEM analysis were structural disorganization of *S. aureus* the cellular membrane 3 hrs and 6 hrs after exposure to the bacteriocin of *Pseudomonas aeruginosa*. Bacteriocins with broad-scale antimicrobial activity can be thought as promising natural antimicrobials for many industrial applications in this manner. Especially, human health and food industries have been dominated the related studies and many prosperous improvements have been done up to date. Bacteriocins have a great potential in human health applications when compared to the traditional antibiotics. Their low-toxicity, high target-specific affect mechanism, presence of various types in nature and effectiveness at nanomolar concentrations are the main advantages of bacteriocins.

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