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# ISOLATION, PRODUCTION AND CHARACTERIZATION OF BACTERIOCINS PRODUCED BY STRAINS FROM INDIGENOUS ENVIRONMENTS

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**Abstract**

About 300 bacterial strains (belonging to different genera) were isolated from different indigenous ecological niches. The sources include food (milk and yogurt), water (drinking water and waste water), soil (garden), stool (healthy infants) and clinical samples. The isolates were screened for their bacteriocin production where 54.3% were found as bacteriocin producers. Among them, Lactobacilli, Pseudomonas, Proteus and Staphylococci displayed good potential of bacteriocin production. *Pseudomonas aeruginosa* SA 188 (a garden soil isolate) was grown in Brain Heart infusion broth of pH 6-7 at $29^0$C overnight (optimum conditions for bacteriocin production). The possibility of bacteriophage activity was also excluded. Partial purification of this pyocin SA188 was achieved after 70% ammonium sulphate precipitation. The bacteriocin titer was calculated to be 640AU/ml.This pyocin did not respond to proteolytic and lipolytic enzymes. It retained its activity at $100^0$C for 15 minutes and pH range of 4 to 10. Surfactants such as SDS could enhance the production of bacteriocin while organic solvents and metal salts had no effect on its production profile.

## Introduction

Antagonistic substances have been reported from prokaryotic and eukaryotic cells as an effort to contain the other organisms living in the same ecological niches for competitive antagonistic inhibition. The production of these substances is a natural defense biological strategy. Bacteriocins are the protein antibiotics produced by bacteria against the closely related strains. However, their broad spectrum inhibitory activity is now well established against different bacteria, certain viruses, fungi and even protozoa. These reports offer multi-facet beneficial activities in clinico-medical practice and pharmaceutical industry in particular (Nes, 2011).

Production of bacteriocin is wide spread in nature. Interestingly, a single species has been reported to produce 10 to 100 different types of bacteriocins (Webster, 1991; James *et al*., 2002). Bacteriocins of *Pseudomonas aeruginosa* presents an example of such excellence as >90% of its strains produce bacteriocins called Pyocins (Michel-Briand & Baysse 2002; Waite & Curtis 2009; Ling *et al*., 2010).

Lactic acid bacteria (LAB) the Gram positive have gained importance because of their bacteriocin production ability with broad spectrum of inhibitory activity and their potential use in food preservation and health care products. Nisin and Pediocin are the well studied bacteriocin products of lactic acid bacilli and are widely used in the food industry for over the past 40 years (Rajaram *et al*., 2010; Vamanu &Vamanu, 2010).The role of bacteriocins as probiotic has also been well documented and in this connection Lactic acid bacilli and Bifidobacterium are gaining credibility for research. A growing focus of research has been on the use of these antimicrobial proteins for treatment of human and plant diseases (Galvez *et al*., 2007; Nes, 2011).

The objective of the present study was to investigate the potential of diverse indigenous bacteria to produce bacteriocin, bacteriocin like inhibitory substances (BLIS), optimization of conditions for production of bacteriocin, their partial purification and characterization.

## Materials and Methods

**Collection of indigenous microorganisms from different environments:** About 335 bacterial strains were isolated from different environmental sources such as food, water, soil, stool and clinical samples (Table 1 ).

**Isolation and identification of organisms:** The organisms were isolated on appropriate media and identified on the basis of morphological and biochemical characterization as per Bergey`s manual of systematic bacteriology (Holt *et al*., 1994).

**Screening of organisms for their bacteriocinogenic potential:** The antibacterial activity of the isolates was detected by Agar well assay (Kang & Lee 2005), cross streak procedure (Pugsley & Oudega 1987 ), agar spot method (Padilla *et al.*, 2002), and stab- overlay method (Rasool *et al*.,1996 ).The antibacterial spectrum was tested against 12 different bacterial strains representing gram positive and the gram negative.

**Selection of best bacteriocin producer and the suitably sensitive organism:** On the basis of bioactivity, *Pseudomonas aeruginosa* SA188, a garden soil isolate was selected as bacteriocin producer for the follow up studies. This strain was re-identified by different tests while final identification was done by using API 20NE kit (Biomerieux, France). *Staphylococcus aureus* SA 84 was selected as the indicator strain for further studies.

**Optimization of culture conditions:** The selected producer strain *Pseudomonas aeruginosa* SA188 was subjected to different culture conditions for maximum production of bacteriocins. Accordingly, the selected strain was grown in different culture media: (Nutrient broth (NB), Trypticase soya broth (TSB), Brain heart infusion broth (BHI), Lactose broth (LB), Luria basal broth (LBB) and Pseudo agar base (PAB). All the media were obtained from Oxoid, UK. Similarly, different incubation temperatures ($4^o$C $10^o$C, $29^o$C, $37^o$C & $40^o$C), incubation period (5, 10, 15, 20, 24, 36, 48 & 72 hours) and pH range of 2-14 were tried to optimize the bacteriocin production. The activity was measured by agar well diffusion assay (Ogunbanwo *et al*., 2003).

**Table 1. Collection of bacterial isolates from different habitats.**

| Name of organism | Food | Water | Soil | Stool | Clinical | Total. |
|---|---|---|---|---|---|---|
| | (No. of isolates) | | | | | |
| *Acinetobacter* spp. | - | 4 | 3 | - | 09 | 16 |
| *Bacillus* spp. | - | - | 8 | - | - | 08 |
| *Corynebacterium* spp. | - | - | - | - | 09 | 09 |
| *Enterobacter* spp. | - | 03 | 01 | - | 08 | 12 |
| *Enterococcus* spp. | - | 09 | - | - | 07 | 16 |
| *Escherichia coli* | - | 07 | 02 | - | 15 | 24 |
| *Klebsiella pneumoniae* | - | 03 | - | - | 18 | 21 |
| *Lactobacillus* spp. | 44 | - | - | 07 | - | 51 |
| *Listeria monocytogenes* | 01 | - | - | - | - | 01 |
| *Proteus* spp. | - | - | - | - | 24 | 24 |
| *Pseudomonas* spp. | - | 09 | 19 | - | 40 | 68 |
| *Salmonella* spp. | - | 08 | - | - | 08 | 16 |
| *Serratia* spp. | - | - | - | - | 06 | 06 |
| *Shigella* spp. | - | 05 | - | - | 06 | 11 |
| *Staphylococcus* spp. | - | 07 | 05 | - | 30 | 42 |
| *Streptococcus* spp. | - | - | - | - | 10 | 10 |
| **Total** | 45 | 50 | 38 | 07 | 190 | 335 |

**Bacteriocin preparation:** After optimization, *Ps.aeruginosa* SA188 was grown in BHI at 29$^{o}$C for 18 hours and the cells were separated by centrifugation at 6000 X g for 30 minutes at 4$^{o}$C.The cell free supernatant(CFS) was adjusted to pH 7.0 and was filter sterilized by a 0.45 µm pore size membrane filter. This crude bacteriocin preparation was partially purified by 70% Ammonium sulphate precipitation at 4$^{o}$C. The precipitate was sedimented by centrifugation at 6000 X g for 45 minutes at 4$^{o}$C. The resulting pellet was suspended in 50mM Sodium Phosphate Buffer of pH 7.0 and referred as partially purified bacteriocin preparation (Harris, 1989; Jabeen *et al*., 2009).The activity units (AU) of both crude and the partially purified bacteriocin preparations were determined by agar well diffusion method. The bacteriocin titer was expressed as activity unit, or arbitrary units/ml. (AU/ml) (Rajaram *et al*., 2010).

**Detection of lytic bacteriophages:** The possibility of either viable or defective phage particle present in the supernatant of producer strain and responsible for inhibitory activity was ruled out by using reverse-side technique (Iqbal *et al*., 1999).

**Characterization of bacteriocin:** The effects of different physical and chemical factors on the stability of bacteriocin preparation were also monitored. For this, bacteriocin preparation was subjected to treatment by different enzymes ( protease, proteinase K, trypsin, papain and lipase (Sigma), varying temperatures (-20$^{o}$C , 4$^{o}$C, 60$^{o}$C, 80$^{o}$C, 100$^{o}$C and 121$^{o}$C (at 15 psi for 15min.), pH range of 2-14, organic solvents (acetone, butanol, ethanol, methanol, propanol and chloroform), heavy metal salts ($AgNO_3$, $BaCl_2$, $CuSO_4$, $FeSO_4$, $MnCl_2$,and $NiSO_4$(Merck) and surfactants (Tween 20, Tween80, SDS (Merck) and EDTA (Sigma). The post treatment bioactivity was assessed by agar well diffusion method (Prasad *et al*., 2005: X ie *et al*., 2009).

**Results and Discussion**

Bacteriocinogenesis and the term `Colicins` were first coined about 90 years ago and today all major groups of bacteria including both the gram negative and the gram positive (even some members of archaea) have been reported to produce these toxic proteins. The bacteriocin producing bacteria are not limited to any particular habitat; they have been isolated from a variety of ecological sites including soil, water, food and human body (Shand & Leyva, 2008; Gillor *et al*., 2008). Bacteriocins show great diversity in their structures and bioactivity. They can be simple, small, low molecular weight peptides e.g. Nisin to high molecular weight proteins e.g., Colicins (Dykes, 1995). Similarly, their bioactivity is exerted mostly against closely related strains but some can target a broad range of species and genera as well (Daw & Falkiner, 1996).The present study is an attempt to isolate bacteriocin producing strains from different habitats.

**Isolation and identification of indigenous bacteria from different habitats:** Different habitats (food, water, soil, stool and clinical samples) were selected for the isolation of bacteria for this study (Table 1). A total of 335 bacterial strains were isolated comprising both gram positive and gram negative bacteria. Among gram positive bacteria *Lactobacillus* spp., and *Staphylococcus* spp., were found predominant. However, among gram negatives *Pseudomonas* spp., *Escherichia coli*, *Proteus* spp., and *Klebsiella pneumoniae* constituted the main isolates (Table 1).

**Bacteriocinogenic potential of the isolated bacteria:** The isolated organisms were screened for their bioactivity against other bacteria. The overall inhibitory spectrum revealed that 54.3% (182/335) of all the isolates exhibited

inhibitory effect against 20% of the bacteria used as the indicator (Table 2). This finding is supported by previous studies where high percentage of bacteriocin producers was reported from different groups of bacteria (Webster, 1991; James *et al.,* 2002). Klaenhammer (1993) recognized 99% of all the bacteria as producer of minimum one type of bacteriocin if they were checked against suitable indicators. In the present study greater number of organisms isolated from clinical specimens exhibited production of bacteriocins. This finding is in agreement with the study of Blinkova (2008) who reported 60% bacteriocin producers from clinical materials. Moreover, *S.aureus* and *Ps.aeruginosa* were the predominant producers with potent and broad spectrum antimicrobial activity. In our study, the organisms which exhibited good potential of bacteriocin production mainly belonged to genera including *Lactobacillus* (82%), *Staphylococcus* (47%), *Proteus* (56%) and *Pseudomonas* (76%). Generally the inhibitory spectrum was more pronounced against most of the gram positive organisms as compared to gram negative bacteria. This might be due to the presence of more receptors for bacteriocin adsorption in peptidoglycan based cell wall of Gram positive bacteria (Padilla *et al*., 2002).

The role of lactocin associated Lactobacilli as antagonists against wide range of bacteria is well studied (Rajaram *et al.,* 2010). Most of the bacteriocins by lactic acid bacilli group members are regarded as GRAS (generally regarded as safe) therefore, during the last few decades, interest has increased in the use of bacteriocins of Lactobacilli for the preservation of food, as probiotic and in the control of diseases (Mojgani *et al.,* 2009). Lactobacilli in the present study were collected from food (milk and yogurt) and from stool samples of healthy infants. These isolates generally showed appreciable level of inhibition of gram positive bacteria however bioactivity against the gram negative was shown by a few strains only (Table 2).

*Staphylococcus aureus*, the gram positive cocci also revealed a good inhibitory spectrum against many bacteria including a food pathogen *Listeria monocytogenes* in present study. It has been shown that bacteriocin production by *Staphylococcus* spp., shows diversity in function and spectrum of inhibition of bacteria, fungi and even viruses (Netz *et al.,* 2001; Saeed *et al.,* 2005).

The present study highlights the isolation of appreciable number of Pseudomonads. These strains were isolated from different sources (13.2% from water, 27.9% from soil, 58.8% from clinical specimens). The isolates of soil and clinical origin exhibited higher potential of bacteriocinogenesis. Bacteriocins by these isolates inhibited the growth of a number of gram positive organisms like *S.aureus, S.pyogenes, Listeria monocytogenes;* however *E.coli, Klebsiella pneumoniae, Shigella dysenteriae, Pseudomonas aeruginosa* and *Proteus mirabilis* were also found susceptible. It is well established that these *Pseudomonas* bacteriocins manifest broad spectrum bioactivity (Akinyemi *et al.,* 2000; Parret & De Mot, 2000 and Iwalokun *et al.,* 2006).

Proteus species, (Gram negative) are well known for causing urinary tract and nosocomial infections. These isolates were also screened for their bacteriocin mediated bioactivity. Interestingly, a strong bioactivity was manifested against gram positive and gram negative bacteria including *S. aureus* and *Listeria monocytogenes* by proteus bacteriocins.These findings are well supported with the findings of Kusek & Herman (1980), who demonstrated significant bacteriocin production by clinical Proteus isolates.

**Selection of representative bacteriocin producer and the indicator strains:** On the basis of screening for antibacterial potential of indigenous organisms, *Pseudomonas aeruginosa* SA 188 (a garden soil isolate) was selected as the bacteriocin producer while *Staphylococcus aurues* SA 84 (a clinical isolate) was selected as the indicator strain.

**Optimization of conditions for bacteriocin production:** The optimization of conditions for maximum production of bacteriocin carries significance in research as well as for cost efficacy. Although, the bacteriocinogenesis is a genetically controlled phenomenon but certain factors also influence the bacteriocin production. The factors include composition of medium (Laulova, 1992), pH of medium (Nilsen *et al.,* 1998), incubation time and temperature (De Vuyst *et al.,* 1996). The bacteriocin production varies with a change in these factors (Delgado *et al*., 2007).In order to determine the suitable nutrient medium for the maximum production of bacteriocin, the selected producer strain *Ps.aeruginosa* SA188 was inoculated in different media. Brain heart infusion (an enriched medium) was ultimately found to be of choice as growth results indicated highest yield of bacteriocin i.e., 640AU/ml. This finding correlates with previous studies where *Pseudomonas* spp., showed maximum production of bacteriocin when grown in BHI compared to other media (Padilla *et al*., 2002; Saleem *et al*., 2009).Although complex enriched media enhances the bacteriocin production but other factors like sources of Carbon and Nitrogen in medium carry significance (Keren *et al*., 2004; Mataragas *et al*., 2004). The most frequent source of carbon is glucose but lactose, galactose and raffinose are also reported to have significant influence on bacteriocin production (Savadogo *et al*., 2006; Vamanu & Vamanu, 2010).

Bacterial growth and bacteriocin production are also influenced by incubation temperature and pH of the growth medium. Maximum production of bacteriocin by *Ps.aeruginosa* SA 188 was witnessed at pH 6-7 and at 29$^{o}$C for 20 to 24 hours (Table 3). This finding is in contrast with a study which reported 35 $^{o}$C -37 $^{o}$C as optimum temperature for bacteriocin production by clinical *Ps.aeruginosa* (Iwalokun *et al.,* 2006). Saleem *et al.,* (2009) reported that bacteriocin production by soil *Ps.aeruginosa* was optimized at 32$^{o}$C .

**Determination of bacteriocin titer:** The antibacterial titer of both crude and partially purified preparation of Pyocin SA 188 was quantified to be 640AU/ml. However; the zone of inhibition by partially purified preparation was more (in diameter) compared to the inhibition by the crude preparation (Figs. 1&2).

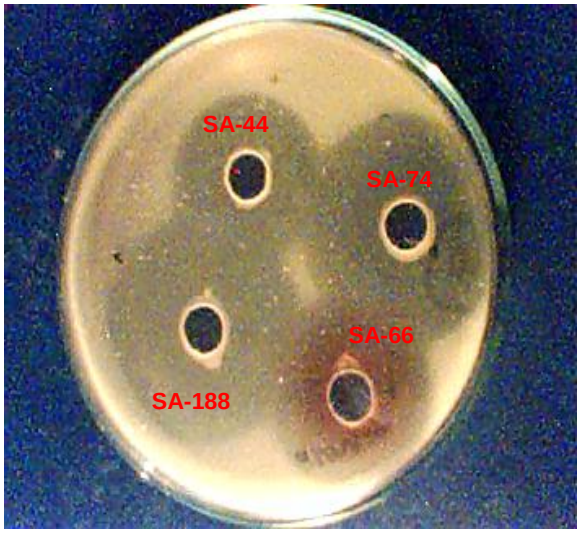
**Table 2. Spectrum of bacteriocin bioactivity by indigenous isolates ( agar well method).**

| Name of organisms screened for bacteriocin production | No. of strains isolated from different environments | % of producers | Indicator organisms | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Gram positive bacteria* | | | | | | Gram negative bacteria* | | | | | |
| | | | *Bacillus subtilis* | *Coynebacterium xerosis* | *Listeria monocytogenes* | *Micrococcus luteus* | *Staphylococcus aureus* | *Streptococcus pyogenes* | *Escherichia coli* | *Klebsiella pneumoniae* | *Proteus mirabilis* | *Pseudomonas aeruginosa* | *Salmonella typhi* | *Shigella dysenteriae* |
| *Acinetobacter* spp. | 16 | 25 | 0/16 | 1/16 | 0/16 | 4/16 | 4/16 | 1/16 | 0/16 | 0/16 | 0/16 | 0/16 | 0/16 | 0/16 |
| *Bacillus* spp. | 08 | 62 | 3/8 | 3/8 | 4/8 | 4/8 | 5/8 | 5/8 | 0/8 | 0/8 | 0/8 | 3/8 | 2/8 | 3/8 |
| *Corynebacterium* spp. | 09 | 22 | 0/9 | 0/9 | 1/9 | 2/9 | 2/9 | 0/9 | 0/9 | 0/9 | 0/9 | 0/9 | 0/9 | 0/9 |
| *Enterobacter* spp. | 12 | 41 | 0/12 | 2/12 | 0/12 | 4/12 | 2/12 | 0/12 | 0/12 | 0/12 | 0/12 | 0/12 | 0/12 | 0/12 |
| *Enterococcus* spp. | 16 | 56 | 0/16 | 3/16 | 4/16 | 8/16 | 5/16 | 4/16 | 0/16 | 0/16 | 0/16 | 0/16 | 0/16 | 0/16 |
| *Escherichia coli* | 24 | 58 | 5/24 | 11/24 | 11/24 | 11/24 | 11/24 | 9/24 | 3/24 | 0/24 | 0/24 | 3/24 | 2/24 | 3/24 |
| *Klebsiella pneumoniae* | 21 | 19 | 0/21 | 1/21 | 0/21 | 2/21 | 2/21 | 0/21 | 0/21 | 0/21 | 0/21 | 0/21 | 0/21 | 0/21 |
| *Lactobacillus* spp. | 51 | 82 | 37/51 | 21/51 | 6/51 | 39/51 | 39/51 | 7/51 | 9/51 | 0/51 | 0/51 | 19/51 | 3/51 | 7/51 |
| *Listeria monocytogenes* | 01 | 100 | 0/1 | 1/1 | 0/1 | 1/1 | 1/1 | 0/1 | 0/1 | 0/1 | 0/1 | 0/1 | 0/1 | 0/1 |
| *Proteus* spp. | 24 | 58 | 7/24 | 5/24 | 3/24 | 12/24 | 14/24 | 6/24 | 11/24 | 5/24 | 0/24 | 5/24 | 0/24 | 4/24 |
| *Pseudomonas* spp. | 68 | 76 | 27/68 | 16/68 | 12/68 | 52/68 | 50/68 | 9/68 | 6/68 | 6/68 | 0/68 | 4/68 | 0/68 | 3/68 |
| *Salmonella* spp. | 16 | 25 | 4/16 | 0/16 | 0/16 | 4/16 | 3/16 | 0/16 | 0/16 | 0/16 | 0/16 | 0/16 | 0/16 | 0/16 |
| *Serratia* spp. | 06 | 16 | 0/6 | 0/6 | 0/6 | 1/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| *Shigella* spp. | 11 | 27 | 2/11 | 1/11 | 0/11 | 3/11 | 1/11 | 0/11 | 0/11 | 0/11 | 0/11 | 0/11 | 0/11 | 0/11 |
| *Staphylococcus* spp. | 42 | 47 | 11/42 | 8/42 | 0/42 | 20/42 | 11/42 | 6/42 | 0/42 | 0/42 | 0/42 | 0/42 | 0/42 | 0/42 |
| *Streptococcus* spp. | 10 | 20 | 0/10 | 0/10 | 0/10 | 2/10 | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 |

*= No. of strains giving bioactivity against indicator organism / total no. of strains tested for bacteriocin production

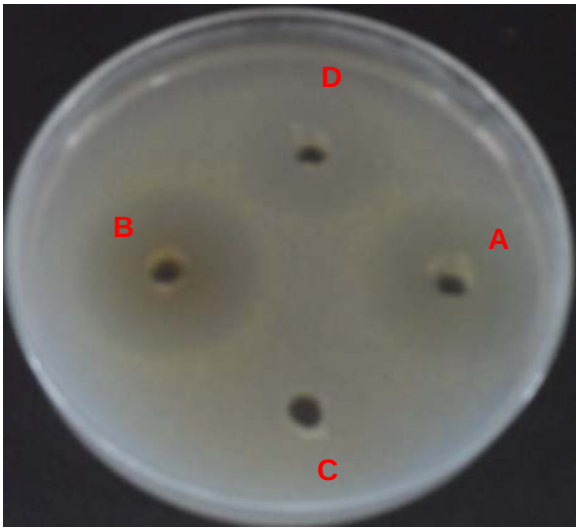
**Table 3. Optimization of conditions for bacteriocin production by *Pseudomonas aeruginosa* SA188.**

| Condition | Bacteriocin activity AU/ml | Condition | Bacteriocin activity AU/ml |
|---|---|---|---|
| **Growth media (at 32°C for 24 hrs.)** | | **Incubation temperature (in BHI medium for 24 hrs.)** | |
| Brain heart infusion broth(BHI) | 640 | 4°C | 0 |
| Nutrient broth (NB) | 320 | 10°C | 0 |
| Luria basal broth (LBB) | 320 | 15°C | 160 |
| Lactose broth (LB) | 320 | 29°C | 640 |
| Trypticase soya broth (TSB) | 320 | 37°C | 320 |
| Pseudo agar base (PAB) | 320 | 40°C | 160 |
| **Incubation time (hrs.) (in BHI medium at 29°C** | | **pH range (in BHI medium at 29°C for 24 hrs.)** | |
| 5 | 160 | 2 | 0 |
| 10 | 160 | 4 | 160 |
| 15 | 320 | 6 | 640 |
| 20 | 640 | 7 | 640 |
| 24 | 640 | 8 | 320 |
| 36 | 320 | 10 | 160 |
| 48 | 320 | 12 | 0 |
| 72 | 160 | 14 | 0 |




SA- 44. *Staphylococcus* sp., SA-74. *Proteus* sp., SA-66. *Pseudomonas* sp., SA-188. *Ps.aeruginosa*

Fig. 1. Indigenous bacteria showing bacteriocin production by agar well method.




A= Cell free supernatant, B= Ammonium sulfate precipitate (70%), C= Supernatant after precipitation, D= Ammonium sulfate precipitate after dialysis (12 KDa cutoff)

Fig. 2. Bioactivity of Pyocin SA188.

**Exclusion of bioactivity by lytic phage activity:** The inhibitory activity by *Ps.aeruginosa SA188* may be due to the lytic bacteriophages as described by Gagliano & Hindsill (1970). In order to exclude out this possibility reverse-side technique was followed. The inhibition zone on the opposite side of agar revealed the ability of bacteriocin to diffuse in the agar medium. On the contrary, the bacteriophages lack this diffusion ability (Iqbal *et al*., 1999).

**Characterization of pyocin SA 188:** The partially purified Pyocin SA188 was characterized with respect to treatment with different enzymes, temperature and pH stability, susceptibility to organic solvents, metal salts and treatment with dissociating agents (Table 4). Proteolytic enzymes (proteases, proteinase K, trypsin & papain) could not digest the pyocin SA188. Similarly Lipase also had no effect on the bioactivity of Pyocin SA 188.These findings are in agreement with the studies on pyocins whereby among three different types of pyocins i.e., S, R and F, only S type was found sensitive to proteolytic enzymes. (Michael-Briand & Baysse, 2002; Denayer *et al.*, 2007,) The resistance to lipase excludes the role of lipid moiety for the bioactivity of this pyocin SA188.

The bioactivity of pyocin SA188 was stable following temperature exposure up to 100°C for 15 minutes but bioactivity was completely lost at 121°C for 15minutes at 15 lbs psi.Our findings are in consonance with the study of Saleem *et al.,* (2009) where pyocin preparation was thermostable even uptill 121°C for 15min.at 15psi.Moreover, partially purified Pyocin SA188 remained fully stable after storage for 8 months at -20°C and four months at 4°C, however, the crude preparation lost its activity after one month at these temperatures. The activity of pyocin SA188 was found to be pH dependant. It retained its activity at pH range of 4 to 10, but lost it activity below 4 and above 10. Similar pattern of pH stability was also demonstrated previously where pyocin activity was decreased at < pH 3 and > pH 10 ranges (Saleem *et al.*, 2009). However, Sano & Kageyama (1981) had reported that Pyocin activity was found stable at pH range of 2 to 14. Pyocin SA 188 retained its bioactivity after treatment with different organic solvents and metal salts. However treatment with surfactants like SDS resulted in enhanced bioactivity which could be due to the increased permeability of the cell membrane of indicator organism or due to the break up of bacteriocin complex into active subunits with enhanced lethal effect (Muriana & Klaenhammer 1991; Graciela *et al.*, 1995).

**Table 4. Physico-chemical characterization of Pyocin SA 188.**

| Enzymes (1mg/ml) | AU/ml | Surfactants (1%) | AU/ml |
|---|---|---|---|
| Protease | 640 | Tween 20 | 640 |
| Trypsin | 640 | Tween 80 | 640 |
| Papain | 640 | EDTA | 640 |
| Proteinase K | 640 | SDS | 1280 |
| Lipase | 640 | | |
| **Temperature (°C)** | | **Organic solvents (5%)** | |
| -20°C (8months) | 640 | Acetone | 320 |
| 4°C (4 months) | 320 | Butanol | 320 |
| 60°C (30min.) | 640 | Ethanol | 320 |
| 80°C (30min.) | 640 | Methanol | 640 |
| 100°C (15min) | 640 | Propanol | 640 |
| 121°C (15min.at 15psi) | 0 | Chloroform | 320 |
| **pH** | | **Metal ions (1mM)** | |
| 2 | 0 | $AgNO_3$ | 640 |
| 4 | 320 | $BaCl_2$ | 640 |
| 6 | 640 | $CuSO_4$ | 640 |
| 7 | 640 | $FeSO_4$ | 640 |
| 8 | 320 | $MnCl_2$ | 640 |
| 10 | 320 | $NiSO_4$ | 640 |
| 12 | 0 | | |
| 14 | 0 | | |

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