Biophysicochemical characterization of Pyocin SA189 produced by
Pseudomonas aeruginosa SA189

Sehar Afshan Naz¹, Nusrat Jabeen¹, Muhammad Sohail², Sheikh Ajaz Rasool²
¹Department of Microbiology, Federal Urdu University of Arts, Science and Technology, Karachi, Pakistan.
²University of Karachi, Karachi, Pakistan.

Submitted: August 29, 2014; Approved: May 2, 2015.

Abstract

Pseudomonas aeruginosa, in spite of being a ubiquitous organism (as it is found in soil, water, and humans), is also an opportunistic pathogen. In order to maintain its diversity in the community, it produces various toxic proteins, known as, bacteriocins. In the present study, pyocin SA189, which is a bacteriocin produced by P. aeruginosa SA189 (isolated from a clinical sample) was characterized. P. aeruginosa SA189, as identified by the conventional and 16S rRNA gene amplification, produced pyocin SA189 of molecular weight of 66 k Da. The pyocin showed antimicrobial activity against several clinically relevant Gram-positive and Gram-negative bacteria and was substantially stable for wide ranges of temperature and pH. Furthermore, the pyocin also retained its biological activity upon treatment with metal ions, organic solvents, and various proteolytic and lipolytic enzymes. The data from the growth kinetics indicated that the maximum bacteriocin production occurred in the late log phase. Overall, our results signify the potential of pyocin SA189 as a bio-control agent.

Key words: bacteriocin, pyocin, P. aeruginosa.

Introduction

Pseudomonas aeruginosa, during the process of establishment in any habitat-soil, water, plants or human and animal systems synthesizes different antimicrobial weapons in order to dominate over the other competing organisms. Pyocin, among others, is a well-known weapon, which facilitates the bacterium to not only invade, but also to defend its ecological niche (Kerr et al., 2002; Riley et al., 2003). In the first report of its kind, Jacob (1954) described a protease resistant pyocin that was obtained from UV irradiated P. aeruginosa. Further investigations elaborated that it adhered to the cell surface of sensitive bacteria that led to their ultimate killing (Michel-Briand and Baysse, 2002).

Afterwards, it was revealed that pyocin is a high molecular weight bacteriocin, which is produced by certain strains of P. aeruginosa and aids in destruction of other P. aeruginosa strains (Ritchie et al., 2011; Dingemans, 2014). However, it has been argued that the antimicrobial spectrum of pyocin exceeds such definition. Activities against other Pseudomonas species, such as, P. fluorescens, P. putida, Burkholderia cepacia complex as well as strains of Neisseria such as N. meningitidis, N. gonorrhoeae, Haemophilus ducreyi and Campylobacter spp. have also been reported (Williams et al., 2008; Bakkal et al., 2010).

Based on the structure and the mode of action, pyocins may be classified as R, F or S-types. A single strain of P. aeruginosa may produce more than one pyocin type at a particular time. Among 1400 strains of P. aeruginosa, isolated from different sources, more than 90% produced one or more types of pyocins (Riley, 1998). For instance, P. aeruginosa strain PA01 produces three types of pyocins, i.e., R2, F2 and S2 (Parret and De Mot, 2002). Riley (1998) observed that the pyocin types, R and F, are produced by more than 90% of the clinical isolates, and the S-type is produced by more than 70% of the strains.

R-type pyocins are high molecular weight proteins, which are resistant to proteases and nucleases. Eight sub-types of R-type pyocin have been described, which are R-1, C-9, R-2, R-3, R-4, R-5, 21 and 430C (Michel-Briand and
Baysse, 2002). These subtypes differ in specificities for their hosts, however, are similar in their morphological features, antigenic properties, and composition of proteins (Lee et al., 1999). Based on the morphological features, as elucidated by transmission electron microscopy (TEM), and the genetic background, the R-type pyocins tend to bear a close resemblance to the T-even phages (Kageyama et al., 1964). However, the physical and chemical stabilities of R-type pyocins, such as, resistance to acids and proteases, make them different from the phages (Scholl and Martin, 2009). The R-type pyocins destroy the target cells without lysing them, this is in contrast to the mode of killing of bacterial phages, where the phage infection results in lysis of the cells (Strauch et al., 2001). The bactericidal activity of R-type pyocins is characterized by its rapidity and specificity, where every individual pyocin molecule is capable of killing a sensitive cell (Scholl et al., 2009).

F-type pyocins are high molecular weight protease-resistant proteins, which were first described by Takeya et al. (1967). The electron microscopic examination revealed that they have a distinct morphological structure, however, are similar to the phage tails. S-type pyocins are soluble, and sensitive to proteases and heat, these features differentiate them from the R and the F- types. The S-type pyocins are composed of two protein sub-units which remain intact throughout the purification process, but they may be separated by gel-filtration in the presence of urea. The larger subunit is the effector component of the S-type pyocin and possesses the killing ability including a DNase activity. On the other hand, the smaller subunit of the pyocin is the immunity component, which provides immunity to the pyocin-producing strains from the destructive effects of the pyocin produced by themselves (Duport et al., 1995; Ling et al., 2010). The present study was designed to produce, purify and study bio-physicochemical characterization of a pyocin produced by an indigenously isolated clinical strain of P. aeruginosa, SA189, which also bears antagonistic activity against the bacterial pathogens.

Materials and Methods

Selection and identification of bacteriocin producing strain

P. aeruginosa SA 189 was originally isolated from a pus sample, which was obtained from a hospitalized patient. The strain has already been reported for its antibacterial activity (Naz and Rasool, 2013). The selected strain, for the current study, was identified by 16S rDNA gene amplification (Spilker et al., 2004) using forward and reverse primers, PA-SS-F “GGGGGATCTTCGGACCTCA” and PA-SS-R “TCCTTAGAGTGCCCACCCG”, respectively.

Inhibitory spectrum of Pyocin SA189

For determination of the inhibitory spectrum of pyocin SA189, several clinical bacterial strains were tested by agar well diffusion assay (Jabeen et al., 2009; Naz and Rasool, 2013). Briefly in this method, the cell free supernatant (CFS) from a 24 hour culture of P. aeruginosa SA189 was filter sterilized using a membrane filter of 0.45μm pore size. A volume of 100 μL of the CFS was poured onto a 7-mm diameter well in nutrient agar plates that were previously seeded with the indicator bacteria (approximately 1 x 10⁶ cfu/mL). The plates were incubated overnight at 37 °C. A clear zone of inhibition surrounding the well of test organism was considered to be positive for the test (Table 1).

Optimization of culture conditions and their kinetics

In order to achieve the maximum production of bacteriocin from P. aeruginosa SA189, the culture was inoculated in different growth media (i.e., nutrient broth, trypticase soya broth, brain heart infusion broth (BHI), lactose broth, Luria-basal broth and pseudo agar base) with varied incubation periods (10, 15, 20, 24, 36, 48 and 72 h) and temperatures (29, 37 and 40 °C for 24 h). The bioactivity of the cell free supernatants of P. aeruginosa SA189 was measured by agar well diffusion assay (AWDA) using Staphylococcus aureus SA 84 as the indicator strain. The inhibitory strength (bacteriocin titer) was expressed as arbitrary units (AU/mL) or activity units/mL (Rajaram et al., 2010). The kinetics of bacteriocin production by P. aeruginosa SA189 was studied by growing the producer strain under optimum conditions for at least 24 h. The samples were collected and studied for the activity of pyocins produced under optimized conditions.

Table 1 - Inhibitory spectrum of Pyocin SA189.

| Indicator strains                  | No of strains tested | Average Zone of inhibition (mm) |
|-----------------------------------|----------------------|---------------------------------|
| Acinetobacter lwoffi              | 6                    | 0                               |
| Bacillus subtilis                 | 5                    | 12                              |
| B. cereus                         | 7                    | 10                              |
| Corynebacterium xerosis           | 4                    | 11                              |
| Enterobacter aerogenes            | 8                    | 0                               |
| Enterococcus faecalis             | 6                    | 17                              |
| Escherichia coli                  | 10                   | 14                              |
| Klebsiella pneumoniae             | 5                    | 14                              |
| Listeria monocytogenes            | 1                    | 17                              |
| Proteus mirabilis                 | 6                    | 0                               |
| Pseudomonas aeruginosa            | 7                    | 9                               |
| Salmonella typhi                  | 8                    | 0                               |
| Serratia marcescens               | 2                    | 0                               |
| Shigella dysenteriae              | 8                    | 0                               |
| Staphylococcus aureus             | 16                   | 26                              |
| E. coli                           | 6                    | 20                              |
| Streptococcus pyogenes            | 6                    | 12                              |
| S. pneumoniae                     | 2                    | 10                              |
| Micrococcus luteus                | 5                    | 22                              |
selected after each hour to record the optical density at 600 nm. Thereafter, the samples were centrifuged and the cell free supernatant of *P. aeruginosa* SA189 was used to determine the activity in units/mL (Jabeen et al., 2009)

**Preparation and purification of Pyocin SA189**

After optimization of culture conditions, *P. aeruginosa* SA189 was grown in BHI broth and was incubated at 37 °C for 24 hours. The following day, the culture broth was subjected to centrifugation at 10,000 x g for 30 min at 4 °C for separation of bacterial cells. The supernatant was filter sterilized by passing through 0.45 μm pore sized filter membrane (Millipore, MA, USA) and was concentrated to 3 to 5-fold using a pre-chilled (4 °C) rotary evaporator (Buschi, Germany). This cell free supernatant (CFS) was referred to as the ‘crude bacteriocin preparation’. This CFS was further partially purified by ammonium sulphate precipitation (Harris, 1989). In order to achieve the maximum saturation of pyocin SA189, different concentrations (50%, 60%, 70% and 80%) of ammonium sulphate were added by constant agitation at 4 °C and precipitates were recovered by centrifugation (10,000 x g for 30 min at 4 °C). The resulting pellets were re-suspended in 50 mM sodium phosphate buffer (pH 7.0) and were designated as ‘partially purified pyocin’ (Jabeen et al., 2009). For removal of the salt from the partially purified bacteriocin, ultra-filtration was done through a pre-treated dialysis tubing of 12 kDa cutoff size (Harris, 1989). The dialyzed bacteriocin was further subjected to gel chromatography on a Sephadex G-75 column of dimensions, 30 x 1.5 cm (Amersham Pharmacia Biotech, USA). The column was pre-equilibrated and the suspension was eluted with 50 mM Sodium phosphate buffer of pH 7.0. The flow rate was maintained at 0.2 mL/min and the eluates were subjected to absorbance measurement at 280 nm. The active fractions, thus obtained, were collected and pooled for assay of inhibitory activity (DeCourcy, 2004). The bioactivity of pyocin SA189 was analyzed at each point of purification by agar well diffusion assay (AWDA), in terms of AU/mL. After every step of purification, protein concentration was measured (Lowry et al., 1951).

**Molecular weight estimation of Pyocin SA189 by SDS-PAGE and related antibacterial assay**

The ammonium sulphate precipitate and their active fractions that were obtained after gel filtration chromatography were subjected to SDS-PAGE (10% polyacrylamide gel) analysis using the standard protein marker (range 14.5 kDa to 200 kDa (Sigma)) loaded on a vertical slab gel (BioRad, USA). After the complete run, the gel was cut into two halves. One half of the gel that contained protein sample and standard molecular weight marker was visualized by Coomassie blue staining to visualize protein bands (Jabeen et al., 2007), while the other half of the gel, containing only the sample protein, was treated with a solution of 20% isopropanol (v/v) and 10% acetic acid (v/v) for 2 h followed by washing with distilled water for 4 h. The gel was placed on a nutrient agar plate and overlaid with soft agar (0.6%) containing the indicator strain. The plate was incubated overnight at 37 °C and next day observed for the zone of inhibition (Bhunia et al., 1988).

**Physico-chemical characterization of Pyocin SA189**

Thermostability of pyocin SA189 was determined by exposing the preparations to elevated temperatures, *i.e.*, from 40 °C to 80 °C for 30 min, and to 100 °C and 121 °C for 15 min. After treatment, the residual activity was determined by AWDA (Jabeen et al., 2009; Benreguieg et al., 2013). To determine the temperature and duration of the bacteriocin stability, the preparation was stored at temperatures, 0 °C, 4 °C or -20 °C, and their bioactivity was monitored till one year using AWDA. To assess the effect of different pH levels on its bioactivity, bacteriocin preparation was adjusted to different pH values, ranging between 1-14, with 1N NaOH (Merek) or 1 N HCl (Merek). The samples were incubated at 37 °C for 2 hours, followed by re-adjustment to neutral (7.0) pH and assessment of the bioactivity by AWDA (Vamanu and Vamanu, 2010). Biological stability of pyocin SA189 was performed after giving a treatment with different enzymes including proteases, proteinase K, pepsin, papain and lipase (Sigma, USA) at a final concentration of 1 mg/mL (Vamanu and Vamanu, 2010). Similarly, equal volumes of the pyocin SA189 were mixed separately with pre-chilled (at 4 °C) 10% concentrated preparations of various organic solvents, acetone, ethanol, methanol and chloroform, with 1 mM solutions of metal ions (CaCl2, FeSO4, ZnSO4, MgSO4, BaCl2) and with different surfactants or detergents (at a final concentration of 1%) including sodium dodecyl sulphate (SDS) and ethylene diamine tetra acetic acid (EDTA), Tween 20, and Tween 80 (Sigma, USA). The mixtures were stirred and incubated at 37 °C for 2 hours and further analyzed by AWDA. The sets of respective positive and negative controls were also processed simultaneously in the same way (Rajaram et al., 2010).

**Results and Discussions**

The present study basically focused on the bacteriocin, which is produced by a clinical strain *P. aeruginosa* SA189. This producer strain as well as the indicator strain, *S. aureus* SA84, were previously isolated and reported (Nazi and Rasool, 2013). The identity of the producer strain (*P. aeruginosa* SA189) was confirmed by 16S rDNA gene amplification (conventional PCR, product size 956 bp).

**Antimicrobial spectrum**

Bacteriocins, in general, share a narrow spectrum of antimicrobial activity; however, there are certain bacteriocins that exhibit a broad spectrum antibacterial activity and are also capable of targeting viruses, protozoa and even...
fungi (Rea et al., 2011). The broad spectrum activity of bacteriocins from *Pseudomonas* spp. has widely been reported (Parret and De Mot, 2000). Pyocin SA189 also exhibited bioactivity against a number of sensitive organisms, notably the Gram-positive bacteria including *S. aureus*, *S. pyogenes* and *Listeria monocytogenes*. However, *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella dysenteriae*, *P. aeruginosa* and *Proteus mirabilis* were also found to be susceptible to a certain extent (Table 1). These findings inspired another study where an isolated pyocin, JU-Ch, exhibited significant antagonistic activity against Gram-positive and Gram-negative bacteria along with different fungi (Grewal et al., 2014). Such bioactive potential might be attributed to the presence of high numbers of bacteriocin adsorption receptors in the peptidoglycan-based cell wall of Gram-positive bacteria (Padilla et al., 2002).

**Optimization of conditions for bacteriocin production and their kinetics**

Like other microbial products, bacteriocin production is a genetically regulated phenomenon and is influenced by a number of environmental and nutritional factors such as the composition of medium, incubation time, temperature, and pH (Lucas et al., 2006, Rajaram et al., 2010). In order to obtain maximum production of the pyocin (bacteriocin) from *P. aeruginosa* SA189, the mentioned parameters were optimized in our current study. The results indicated that BHI broth (an enriched medium) enhanced the production of pyocin (data not shown). Enriched medium not only favors the growth of *P. aeruginosa* but also helps the bacterium to yield high titers of bacteriocins (MacKinnon, 2011). The maximum yield of pyocin SA189 was obtained when the organism was cultivated at 37 °C and pH 7.0. Our finding is in concordance with the previous study conducted by Scholl and Martin (2008). We recorded the maximum titer of pyocin SA189 as 640 AU/mL (data not shown).

The analysis of the growth and production kinetics revealed that the pyocin SA189 production varies in different phases of the growth cycle. The protein production is initiated in the early logarithmic phase, reaches the maximum level after 18 to 22 hours (late log phase) and remained constant till the late stationary phase (Figure 1).

**Purification of Pyocin SA189**

Production of pyocin is regulated in such a manner that only a few cells in a population actively produce pyocin. Further, the bacteriocin production can be enhanced by induction with DNA damage treatments, such as ultraviolet irradiation (Higerd et al., 1967), mitomycin C (Kageyama, 1964) or by DNA gyrase inhibiting antibiotics (Brazas and Hancock, 2005). However, in the present study, the pyocin SA189 was harvested by propagation of the respective strain as per the optimum growth requirements followed by partial purification by ammonium sulphate precipitation, of the cell free supernatant (CFS). The salts, in fact, make the proteins resistant to denaturation, proteolysis or bacterial contamination (Harris, 1989). The maximum antagonistic activity, in case of pyocin SA189, was observed to be 1,280 AU/mL in the resolved precipitate with 70% saturation, which is in concordance with the previously reported findings of Scholl and Martin (2008). After ultra-filtration of partially purified pyocin SA189, it was noted that the pyocin almost retained their activity; however, a little loss might be attributed to the adsorption of the bacteriocin on the dialysis membrane. In the following step of conventional gel permeation chromatography, on Sephadex G-75 column, pyocin SA189 depicted the activity to reside in fraction 6, 7 and 8 and the chromatogram also showed a single peak of protein (Figure 2). These find-

---

**Figure 1** - Pyocin SA189 Production during the growth cycle of *P. aeruginosa* SA189.
ings correlate with the study, where pyocin was eluted from the CM Sephadex A-50 (pH 7.2) column as a single protein peak (Al-Shibib et al., 1985). Furthermore, Duport et al. (1995) determined pyocin S3 as a single protein peak upon Sephacryl S-200 gel filtration (Mr of 90,000). In contrast to these results, a protease sensitive S-type pyocin AP41 was observed as a complex of two proteins that were observed as two separate peaks when subjected to gel filtration Sephacryl S 200 column in the presence of 6 M urea (Sano and Kageyama, 1981).

In the protein purification profile of pyocin SA189, the specific activity in CFS was found to be 220.7 AU/mg, which increased up to 752.9 AU/mg after ammonium sulphate precipitation and 1,828.6 AU/mg after gel filtration (Table 2). The final purification that was achieved after gel filtration chromatography was 8.2 folds with 2% recovery. These findings are in accordance with another study which also reported the purification of pyocin with an increase in the specific activity as well as purification up to 434-fold (Al-Shibib et al., 1985). In another study, the specific activity of the pyocin after every step of purification was increased and final recovery after chromatography using Sephacryl S 200 was observed to be 13% (Sano and Kageyama, 1981). Increase in the specific activity was also demonstrated in each purification step for the pyocin 42A (Abdi-Ali et al., 2004).

SDS-PAGE and related bioactivity

The purity of pyocin protein SA189 was further checked by electrophoresis and confirmed by gel overlay technique. Polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulphate (SDS), an anionic detergent, is one of the important procedures, used successfully to characterize proteins with respect to the molecular sizes of their constituent polypeptides. Moreover, in bacteriocin studies, it has been a widely used technique, which is sensitive enough to detect the activity of the bacteriocin directly on the gel. This technique was employed to check the purity of pyocin SA189. Ammonium sulphate precipitates and the active fractions from gel filtration were subjected to 10% acrylamide denaturing gel. Electrophrogram of pyocin SA189 revealed a single band, which corresponded to the molecular mass of approximately 66 kDa. This band also showed bioactivity by gel overlay method (data not shown). The high molecular weight pyocins, particularly the R-type pyocins are well-documented in literature (Ritchie et al., 2011). A study in this relation demonstrated the isolation of five pyocins from clinical isolates, which were resistant to trypsin treatment and had molecular masses within the 54.9-282 Kg/mol. range (Al-Rubiee et al., 1988). Similar protease-resistant pyocins with 20-135 kg/mol. molecular masses have been reported earlier (Al-Shibib et al., 1985). In another study, R-type pyocin, resistant to all tested proteases, depicted a major band of about 30 kDa on SDS-PAGE (Fontoura et al., 2009). On the other hand, a study on protease-sensitive pyocin showed a single band corresponding to molecular weight 62.4 kDa on the SDS-PAGE (Ling et al., 2010).

Physicochemical characterization of pyocin SA189

Different physical and chemical factors may have significant impact on the bioactivity of bacteriocins. The determination of such impacts bears significance for possible application(s) of bacteriocins in food preservation, as probiotics or as chemotherapeutic agents. Interestingly, in the present study, pyocin SA189 was found to be resistant to
the action of almost all the physical and chemical factors tested (Table 3). Thus, as per our analysis, pyocin SA189 could be stated as thermotolerant in nature. Our observations suggested that the pyocin retained its bioactivity even at the autoclaving temperature. Such high thermostability is in contrast to a previous report where R-type pyocin lost activity at a temperature above 60 °C (Al-Rubiee et al., 1988). However, Padilla et al. (2002) found a pyocin to be resistant to proteolytic enzymes as well as high temperatures. Moreover, an antimicrobial peptide produced by *Pseudomonas* spp. also showed resistance to high ranges of temperature (Fontoura et al., 2009). 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 (Sano and Kageyama, 1981; Padilla et al., 2002; Saleem et al., 2009). However, in contrast to our findings, an R-type pyocin demonstrated stability only in a pH range of 6 to 8 (Al-Rubiee et al., 1988).

Besides the physical agents, certain chemicals may modulate the bioactivity of pyocins. The most important finding was the resistance of pyocins SA189 to different proteolytic enzymes (Table 3). Although, this pyocin manifested an apparent proteinaceous nature in various experiments, such as ammonium sulphate precipitation, SDS-PAGE analysis and gel filtration, but when treated with proteolytic enzymes, like proteinase K, proteases, trypsin and papain, it survived digestion and retained its bioactivity. In addition, lipase also had no effect on the activity of this pyocin. These results were not surprising when compared with previous findings on pyocins, where protease-resistant pyocins were obtained from the strains isolated from well water (Padilla et al., 2002), pond water (Fontoura et al., 2009) and clinical materials (Al-Rubiee et al., 1988). Similarly, pyocins that are sensitive to proteases were also reported from different environments (Sano and Kageyama, 1981). The resistance to proteases might be due to the cyclic peptide nature of some of the bacteriocins, which may have unusual amino acids rendering them resistant to hydrolysis by proteolytic enzymes (Bizani and Brandelli, 2002). Exposure of pyocin SA189 to surfactants also resulted in complete retention of their activity. Incubation of pyocin SA189 with various concentrations of organic solvents and metal salts had no effect on its bioactivity. These findings are in accordance with a previously reported study where a pyocin was observed to be resistant to action of all the organic solvents (Saleem et al., 2009).

### Table 3 - Physicochemical characteristics of Pyocin SA189.

| Treatments          | Activity Units (AU/mL) | Treatments          | Activity Units (AU/mL) |
|---------------------|------------------------|---------------------|------------------------|
| **Temperature**     | (Pyocin alone) = 640 AU/mL | **Metal Salts (1 mM)** | (Pyocin alone) = 640 AU/mL |
| 40 °C to 100 °C (30 min) | 640 | BaCl₂ | 640 |
| 121 °C (15 psi 15 min) | 320 | CaCl₂ | 640 |
| 4 °C | 640 | ZnSO₄ | 640 |
| 0 °C-20 °C (till one year) | 640 | FeSO₄ | 640 |
| pH treatment (2 hours) | 640 | MgSO₄ | 640 |
| 2-3 | 320 | Surfactants (1%) | 640 |
| 4-9 | 640 | SDS | 640 |
| 10-11 | 160 | EDTA | 640 |
| 12-14 | 0 | Tween 20 | 640 |
| Protease | 640 | Tween 80 | 640 |
| Proteinase K | 640 | Solvents (10%) | 640 |
| Lipase | 640 | Acetone | 640 |
| Papain | 640 | Chloroform | 640 |
| Trypsin | 640 | Ethanol | 320 |
| | 640 | Methanol | 640 |
References

Abdi-Ali A, Worobec EA, Deezagi A et al. (2004) Cytotoxic effects of pyocin S2 produced by Pseudomonas aeruginosa on the growth of three human cell lines. Can J Microbiol 50:375-381.

Al-Rubiee R, Al-Mudhaifir S, Hassan F et al. (1988) Purification and characterization of pyocins R from Pseudomonas aeruginosa. Folia Microbiol 33:520-524.

Al-Shibah II, Al-Mudhaifir S, Al-Ani M et al. (1985). Purification and characterization of pyocins from Pseudomonas aeruginosa. Folia Microbiol 30:25-29.

Bakka S, Robinson SM, Ordonez CL et al. (2010) Role of bacteriocins in mediating interactions of bacterial isolates taken from cystic fibrosis patients. Microbiol 156:2058-2067.

Benreguiea M, Dalache F, Gacemi B (2013) Characterization of antibacterial activity and potential as probiotic of lactic acid bacteria isolated from goat’s milk in Algeria. J Life Sci 7:802-813.

Bhunia AK, Johnson MC, Ray B (1988) Purification, characterization and antimicrobial spectrum of a bacteriocin produced by Pediococcus acidilactici. J Appl Bacteriol 65:261-268.

Bizani D, Brandelli A (2002) Characterization of a bacteriocin produced by a newly isolated Bacillus sp. strain SA. J Appl Microbiol 93:512-519.

Brazas MD, Hancock RE (2005) Ciprofloxacin induction of a susceptibility determinant in Pseudomonas aeruginosa. Antimicrob Agents Chemother 49:3222-3227.

DeCourcy K (2004) Column chromatography information manual. Franklin Biotechnology Center. Virginia Technology pp. 5-17.

Dingemans J, Ye L, Hildebrand F et al. (2014). The deletion of TonB-dependent receptor genes is part of the genome reduction process that occurs during adaptation of Pseudomonas aeruginosa to the cystic fibrosis lung. Pathog Dis 71:26-38.

Duport C, Bayssé C, Michel-Briand Y (1995) Molecular characterization of pyocin S3, a novel S-type pyocin from Pseudomonas aeruginosa. J Biol Chem 270:8920-8927.

Fontoura R, Spada JC, Silveira ST et al. (2009) Purification and characterization of an antimicrobial peptide produced by Pseudomonas sp. Strain 48. World J Microbiol Biotechnol 25:205-213.

Grewal S, Bhagat M, Vakhlu J (2014) Antimicrobial protein produced by Pseudomonas aeruginosa JU-Ch 1, with a broad spectrum of antimicrobial activity. Biocatal Agri Biotech 8:1290-1305.

Harris ELV (1989) Concentration of the extract: Protein Purification Methods. In: A Practical Approach. E.L.V. Harris and S. Angal (eds) IRL Press, Oxford, pp. 125-172.

Higerd TB, Baechler CA, Berk RS (1967) In vitro and in vivo characterization of pyocin. J Bacteriol 93:1976-1986.

Jabeen N (2007) Bacteriocins for the control of indigenous phytopathogenic bacteria. Ph.D. Thesis. Department of Microbiology, University of Karachi, pp. 49-52.

Jabeen N, Gul H, Subhan SA et al. (2009) Biophysical-chemical characterization of bacteriocin(s) from indigenously isolated Agrobacterium radiobacter NA 6. Pak J Bot 41:3227-3237.

Jacob F (1954) Induced biosynthesis and mode of action of a pyocine, antibiotic produced by Pseudomonas aeruginosa. Ann Inst Pasteur 86:149-160.

Kageyama M, Ikeda K, Egami F (1964) Studies of a pyocin. III. Biological properties of the pyocin. J Biochem 55:59-64.

Kerr B, Riley MA, Feldman MW et al. (2002) Local dispersal promotes biodiversity in a real-life game of rock-paper-scissors. Nature 418:171-174.

Lee FK, Dudas KC, Hanson JA et al. (1999) The R-type pyocin of Pseudomonas aeruginosa is a bacteriophage tail-like particle that contains a single-stranded DNA. Infect Immun 67:717-725.

Ling H, Saeidi N, Rasouliha BH et al. (2010) A predicted S-type pyocin shows a bactericidal activity against clinical Pseudomonas aeruginosa isolates through membrane damage. FEBS Lett 584:3354-3358.

Lowry OH, Rosebrough NJ, Farr AL et al. (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265-275.

Lucas R, Grande MA, Abriouel H et al. (2006) Application of the broad-spectrum bacteriocinenterocin AS-48 to inhibit B. coagulans in canned fruit and vegetable foods. Food Chem Toxicol 44:1774-1781.

MacKinnon EM (2011) Characterizing the pyocin activity of diverse Pseudomonas aeruginosisa isolates, M.Sc. thesis, Department of Molecular Genetics, University of Toronto, Canada.

Michel-Briand AY, Bayssé C (2002) The pyocins of Pseudomonas aeruginosa. Biochimie 84:499-510.

Naz SA, Rasool SA (2013) Isolation, production and characterization of bacteriocins produced by strains from indigenous environments. Pak J Bot 45:261-267.

Padilla C, Lobos O, Brevis P et al. (2002) Effects of the bacteriocin PsVP-10 produced by Pseudomonas sp. on sensitive bacterial strains. De Microbiologia 44:19-23.

Parret AHA, De Mot R (2002) Bacteria killing their own kind: novel bacteriocins of Pseudomonas and other γ-proteobacteria. Trends Microbiol 10:107-112.

Rajaram G, Manivasagan P, Thilagavathi B et al. (2010) Purification and Characterization of a bacteriocin produced by Lactobacillus lactis isolated from marine environment. Adv J Food Sci Technol 2:138-144.

Rea MC, Ross RP, Cotter PD et al. (2011) Classification of bacteriocins from gram positive bacteria. In: Prokaryotic Antimicrobial Peptides. From Genes to Application. Drider, D. and Rebuffat, S. (eds). Springer, Berlin, pp. 29-54.

Riley MA (1998) Molecular mechanisms of bacteriocin evolution. Annu Rev Genet 32:255-278.

Riley MA, Goldstone CM, Wertz JE et al. (2003) A phylogenetic approach to assessing the targets of microbial warfare. J Evol Biol 16:690-697.

Ritchie JM, Greenwich JL, Davis BM et al. (2011) An Escherichia coli O157-specific engineered pyocin prevents and ameliorates infection by E. coli O157:H7 in an animal model of diarrheal disease. Antimicrob Agents Chemother 55:5469-5474.

Saleem F, Ahmed S, Yaqoob Z et al. (2009) Comparative study of two bacteriocins produced by representative indigenous soil bacteria. Pak J Pharma Sci 22:252-258.

Sano Y, Kageyama M (1981) Purification and properties of an S-type pyocin, Pyocin AP41. J Bacteriol 146:733-739.
Scholl D, Martin DW (2008) Antibacterial efficacy of R-type pyocins towards Pseudomonas aeruginosa in a murine peritonitis model. Antimicrob Agents Chemother 52:1647-1652

Scholl D, Cooley M, Williams SR et al. (2009) An Engineered R-Type Pyocin is a highly specific and sensitive bactericidal agent for the food-borne pathogen Escherichia coli O157:H7. Antimicrob Agents Chemother 53:3074-3080.

Spilker T, Coenye T, Vandamme P et al. (2004) PCR-based assay for differentiation of Pseudomonas aeruginosa from other Pseudomonas species recovered from cystic fibrosis patients. J Clin Microbiol 42:2074-2079.

Strauch E, Kaspar H, Schaudin C et al. (2001) Characterization of enterocoliticin, a phage tail-like bacteriocin, and its effect on pathogenic Yersinia enterocolitica strains. Appl Environ Microbiol 67:5634-5642.

Takeya K, Minamishima Y, Amako K et al. (1967) A small rod-shaped pyocin. Virol 31:166-168.

Vamanu E, Vamanu A (2010) The influence of prebiotics on bacteriocin synthesis using the strain Lactobacillus paracasei CMGB16. Afr J Microbiol Res 4:534-537.

Williams S, Gebhart D, Martin D et al. (2008) Retargeting R-typing pyocins to generate novel bactericidal protein complexes. Appl Environ Microbiol 74:3868-3876.

Associate Editor: Elizabeth de Andrade Marques

All the content of the journal, except where otherwise noted, is licensed under a Creative Commons License CC BY-NC.