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ISOLATION AND CHARACTERIZATION OF PYOCIN S2-PRODUCING LOCAL STRAINS OF *Pseudomonas aeruginosa*

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ABSTRACT

Antibiotic resistance is responsible for 1.27 million deaths globally. Pyocin, from *Pseudomonas aeruginosa* is a promising candidate against antibiotic-resistant infections. This study aims at isolating pyocin S2-producing local strains of *Pseudomonas aeruginosa* and investigating the antimicrobial activity of pyocin S2 against *Staphylococcus aureus*, *Staphylococcus* sp., *Escherichia coli*, *Salmonella* sp., *Streptococcus pneumoniae*, and *Vibrio cholerae*. *Pseudomonas aeruginosa* was isolated from environmental and clinical samples by inoculation on cetrimide agar plates and incubating at 37^0C for 24 hours. To detect the presence of pyocin S2 gene in the isolates: DNA extraction, polymerase chain reaction (PCR) using primers specific to pyocin S2 gene, and agarose gel electrophoresis was conducted. Using agar well diffusion assay, the antimicrobial activity of pyocin S2-containing *Pseudomonas aeruginosa* against the aforementioned target pathogens was evaluated. Out of 20 *Pseudomonas aeruginosa isolates* subjected to PCR, 11 contained pyocin S2 gene. Pyocin S2 from *Pseudomonas aeruginosa* A1, B2, CL2, CL8 and C11 produced zone of inhibition (ZOI) when tested against *Escherichia coli* (ZOI = 23.5mm, 17.5mm, 26.0mm, 21.5mm, 29.0mm respectively). However, pyocin S2 from *Pseudomonas aeruginosa* B3, B10, CL3, B19, CL9 and CL6 did not inhibit *Escherichia coli* but suppressed the growth of *Staphylococcus sp.* (ZOI = 21.0mm, 18.5mm, 28.5mm, 23.7mm, 24.5mm and 23.5mm respectively). Also, Pyocin S2 from *Pseudomonas aeruginosa* C11 suppressed the growth of *Vibrio cholerae* (ZOI = 30.7mm), but not the other strains. In conclusion, pyocin S2 from indigenous strains of *Pseudomonas aeruginosa* inhibited the target pathogens. These findings will foster further evaluation of pyocin S2's antimicrobial potential.

Keywords: Antibiotic resistance, Antimicrobial activity, Bacteriocin, *Pseudomonas aeruginosa*, Pyocin S2

INTRODUCTION

Infections derived from pathogenic microorganisms that are resistant to treatments are on the rise globally. An estimate of 1.91 million deaths are attributable to antimicrobial resistance (AMR) and 8.22 million deaths associated with AMR could occur globally in 2050 (Naghavi *et al.*, 2024). In response, a number of therapy techniques, such as the production of bacteriocins, have been examined recently (Meade *et al.*, 2020; Wayah *et al.*, 2024a). Bacteriocins are antimicrobial peptides that bacteria produce to stop other bacteria from growing, especially closely related bacteria (Heilbronner *et al.*, 2021). Bacteriocins help to eradicate rival microorganisms that occupy the same ecological niche and are by-products of the secondary metabolism of many bacterial genera. However, not all strains produce bacteriocins continually because it requires energy and nutrients; in fact, the synthesis of these metabolites is triggered by quorum sensing mechanisms, which are genetic self-regulation systems (García-Curiel, 2021). So far, many bacteriocins have been widely identified with various modes of action as follows: Inducing cytoplasmic membrane permeability (by pore formation), inhibiting cell wall biosynthesis, and interfering with metabolic pathways (Hols *et al.*, 2019).

Pyocins are bacteriocins generated by *Pseudomonas aeruginosa*, a Gram-negative bacterium notorious for causing infections in humans. Pyocins arise from strains of *Pseudomonas aeruginosa* species by pyocinogeny (Atanaskovic *et al.*, 2020). Pyocins, which are proteinaceous antimicrobial compounds that have strong inhibitory activity against other bacterial strains. They also have a lot of potential for use in other applications. They are highly sought after for use in bioengineering and antibacterial applications because of their tremendous killing capacity (Zinke *et al.*, 2022). They are categorized according to their size, mode of action, and genetic organization. Pyocins generally work by targeting and disrupting certain parts of bacterial cells, like DNA or cell membranes, which causes cell death. Depending on the type of pyocin, their mechanism of action can vary. Pyocins have a high degree of target specificity, affecting mainly closely related bacterial strains along with other *Pseudomonas* species. This specificity can help to eliminate certain pathogens without damaging beneficial bacteria. Pyocins may be used to control *Pseudomonas aeruginosa* infections. Because of their antibacterial qualities, they might also find use in biotechnology, agriculture, and food preservation. Due to evolutionary pressures, pyocins have the potential to quickly evolve in order to combat the mechanisms of bacterial resistance. They come in a variety of forms and may have different specificities and modes of action (Cornelissen *et al.*, 2011; Six *et al.*, 2021).

Pyocin S2 is a specific type of pyocin produced by *Pseudomonas aeruginosa*. Pyocin S2 has a long, filamentous structure composed of a narrow tail and a contractile sheath reminiscent of bacteriophages. It is classified as a contractile-tail bacteriocin, distinguishing it from other types of pyocins. Pyocin S2 functions by attaching to the surface of susceptible cells using its narrow tail structure (Goult *et al.*, 2024). Upon attachment, the contractile sheath contracts rapidly, puncturing the target cell's membrane and leading to cell lysis and death. Pyocin S2 exhibits potent antimicrobial activity against other strains of *Pseudomonas aeruginosa*, making it a potential candidate for combatting *Pseudomonas aeruginosa* infections, particularly in clinical settings. The production of pyocin S2 is regulated by specific genes within the genome of *Pseudomonas aeruginosa*. Understanding the genetic regulation of pyocin S2 production can provide insights into its expression and potential applications (Goult *et al.*, 2024).

Based on available literatures, indigenous strains of *Pseudomonas aeruginosa* have not been explored for the production of pyocin S2 hence the need for this study. The aim of this study was to isolate pyocin S2-producing local strains of *Pseudomonas aeruginosa* and investigate the antimicrobial activity of pyocin S2 against *Staphylococcus aureus*, *Staphylococcus* sp., *Escherichia coli*, *Salmonella* sp., *Streptococcus pneumoniae*, and *Vibrio cholerae*.

MATERIALS AND METHODS

Isolation of Pyocin S2-Producing *Pseudomonas aeruginosa*

Clinical Samples

Isolates of *Pseudomonas aeruginosa* gotten from 25 wound samples were collected from the laboratories of two major hospitals located in Kaduna metropolis namely, St Gerard Catholic Hospital and Barau Dikko Teaching Hospital using sterile cotton swap sticks according to the procedure stated by Adedeji *et al*. (2007) and transported to the post graduate laboratory of the Department of Biochemistry, Kaduna State University for reconfirmation. Each of the cotton swaps containing clinical isolates of *Pseudomonas aeruginosa* were streaked on freshly prepared cetrimide agar plates and incubated at 37^0C for 24 hours. A loopful of the pure isolates was picked from the plates with observed growth and transferred into a freshly prepared nutrient broth solution and incubated at 37^0C for 24 hours. 0.5ml of the inoculums and 0.5ml of glycerol were transferred into eppenddorf tubes then stored at -4^0C as described by Ezeador *et al.* (2020) with slight modification.

Soil Samples

Soil samples were collected according to the procedure stated by Prashanthi *et al.* (2021) with slight modification at 25 various locations on Zitti Street of Sabon Tasha, Chikun LGA and 20 different locations at the school premises of Kaduna State University, Kaduna North LGA of Kaduna state. From each site 10g of soil was taken at 10cm depth to avoid contamination with other surfaces and destruction of microbes by various human activities. Each of the samples was collected into sterile sample bottles and transported to the post graduate laboratory of the Department of Biochemistry, Kaduna State University. 1g of each soil sample was transferred into sterile test tubes and 10ml of sterile water was added and vortexed to make soil solutions. A loopful of each of the soil solutions was streaked on freshly prepared cetrimide agar plates and then incubated at 37^0C for 24 hours. A loopful of isolate was picked from the plates with growth and transferred into a sterile nutrient broth solution and incubated at 37^0C for 24 hours. 0.5ml of the inoculums and 0.5ml of glycerol were transferred into eppenddorf tubes then stored at -4^0C as described by Ezeador *et al.* (2020) with slight modifications.

Water Samples

Water collection sites include Malali, Rigachuku, Sabon Tasha and Kaduna state university in Kaduna state metropolis. The water samples were collected into sterile sample bottles and transported to the post graduate laboratory of the Department of Biochemistry, Kaduna State University. A loopful of each of the soil solutions was streaked on freshly prepared cetrimide agar plates and then incubated at 37^0C for 24 hours. A loopful of isolate was picked from the plates with growth and transferred into freshly prepared nutrient broth solution and incubated at 37^0C for 24 hours. 0.5ml of the inoculums and 0.5ml of glycerol were transferred into eppenddorf tubes then stored at -4^0C as described by Ezeador *et al.* (2020) with slight modification.

Skin Swap Samples

Moist sterile cotton swap sticks was rubbed on the skin of 20 individuals to obtain their various skin swap samples and transported to the post graduate laboratory of the Department of Biochemistry, Kaduna State University. Each moist cotton swap stick containing skin swap samples was streaked on freshly prepared cetrimide agar plates and then incubated at 37^0C for 24 hours. Plates were observed for growth.

Morphological and Biochemical Characterization of *Pseudomonas aeruginosa* isolates.

Stored isolates were characterized using morphological and some biochemical tests such as: Gram staining, methyl red test, citrate utilization test, Voges Proskauer test, indole test, catalase test, and coagulase test as described by Chauhan (2020).

Detection of pyocin S2 Gene in *Pseudomonas aeruginosa* Isolates.

DNA Extraction of *Pseudomonas aeruginosa*

An amount of 5ml of *Pseudomonas aeruginosa* culture was grown over night in nutrient broth. Bacterial cells were harvested by centrifugation at 5000rpm for 5 minutes for pellet. The supernatant was discarded and the pellet re-suspend in 1ml sterile buffer.

A quantity of 200µl of sample was dispensed into a tube of 1.5ml. Lysis buffer of 400µl was measured and added to the sample and 10µl of proteinase K was also added to the tube and incubated at 55°C for 60 minutes. About 400µl of equilibrated phenol at pH 7.8 was added after incubation, vortexed, and centrifuged at 12000rpm for 5 minutes. The supernatant was collected into another tube and 700µl of Chloroform: Isoamyl alcohol (24:1) (672µl Chloroform: 28µl Isoamyl alcohol) was added, mixed well and centrifuged at 12000rpm for 5 minute and the supernatant layer was transferred into a fresh tube. A quantity of 40µl of 3M sodium acetate at pH 5.2 was then added. After which 400µl of 100% ethanol was added and incubated at -20°C for over an hour. The solution was centrifuged for 15 minutes at 4°C at 14000rpm to obtain pellets of the DNA. The supernatant was carefully removed without disturbing the DNA pellet. Again, 150µl of 70% cold ethanol was added and the sample centrifuged at 4°C for 2 minutes at 14000rpm and the supernatant was discarded. The DNA pellet was dried at room temperature for 5-10 minutes and resuspended in 100µl of molecular grade water by pipetting up and down. Finally, the resuspended DNA pellet was centrifuged briefly to collect the sample and the tube placed on ice.

Primer Sequence design and synthesis

The primer utilized in this study was designed using the Primer-BLAST tool available on the NCBI website. Before synthesis, the quality of the primer was verified using the Oligo-analyzer and Primer-Stat then transferred for synthesis (Shehu *et al*., 2024).

Polymerase Chain Reaction (PCR)

For PCR amplification, a total reaction volume of 25µl was prepared, comprising 12.5µl of 2 X Mastermix with Standard buffer, 0.5µl of the forward primer, 0.5µl of the reverse primer, 10µl of nuclease-free water, and 2µl of DNA lysate. The polymerase chain reaction set up involved the addition of the mastermix, forward and reverses primers and the extracted DNA into a PCR vial. Amplification commenced with an

initial denaturation at 94°C for 30 seconds, followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at temperatures of 55°C, 68°C, and 68°C for 1minute each, corresponding to pyocin S2. Extension at 68°C for 5 minutes and final extension procedure was done for 4 minutes at 10°C (Wayah *et al.*, 2024b).

Preparation of Agarose Gel for Electrophoresis

30ml of 1 X TRIS Borate EDTA buffer was measured using the measuring cylinder and transferred into a beaker, 1% Agarose concentration, (0.3g dissolved into the buffer was used to make the gel). Microwave was used to heat the mixture to form a clear colourless solution and allowed to cool slightly; 1µL of ethidium bromide was added and was shaken gently until it mixed properly. The gel block was covered at both ends with masking tape. The solution was poured into the block and allow for some few minutes to solidify. The tapes were removed and the gel block transferred into a tank filled with 1 X TAE buffer and the comb removed. Prepared Genomic DNA samples or PCR products were loaded into the wells and DNA ladder loaded into the first well to serve as the control.

Electrophoresis was carried out at 80-volt 400mA for 25minutes using the agarose gel electrophoretic machine and viewed under the blue light gel transilluminator. M represent 1000 bp marker ladder used to estimate the Genomic DNA bands sizes.

Investigation of the Antimicrobial Activity of Pyocin S2

Production of crude pyocin

An amount of 1ml inoculums of each *Pseudomonas aeruginosa* isolate was added to 9ml of freshly prepared nutrient broth and incubated at 37^0C for 24 hours. The 24 hours cultures were centrifuged at 5000rpm for 10 minutes and the cell free supernatants were carefully decanted into sterile test tubes serving as crude pyocin (Sethi *et al.,* 2013).

Preparation of Target Bacteria Pathogens (Indicator)

A milliliter of inoculums of each target bacteria pathogens was added to 9ml of freshly prepared nutrient broth and incubated at 37^0C for 24 hours. The 24 hours cultures were centrifuged at 5000 rpm for 15 minutes and the cell free supernatant was disposed leaving the cell pellets. The cell pellets were re-suspended in normal saline solution and their optical densities (OD) adjusted to 0.1 (Sethi *et al.*, 2013).

Agar well diffusion assay

A sterile cotton swap stick was dipped in each indicator solution and spread on freshly prepared nutrient agar plate. Wells were made in each inoculated plate using a sterile cork borer of 5mm diameter. About 25µl of each crude pyocin was carefully poured into three out of four wells bored and sterile nutrient broth was poured into the fourth well serving as control and the plates were incubated for 24 hours for 37^0C. After 24 hours the zone of inhibition (ZOI) was observed and measured on plates that showed antimicrobial activity of pyocin S2 on the target pathogens (Sethi *et al.*, 2013).

RESULTS AND DISCUSSION

Results

Isolates of Pseudomonas aeruginosa from clinical and environmental samples

Out of the 25 clinical samples that were inoculated, 19 of them grew on the Cetrimide agar plates but only 11 grew giving a green coloration on the plates confirming them as *Pseudomonas aeruginosa*. These 11 isolates were labeled CL1 to CL11 to differentiate them from other *Pseudomonas aeruginosa* isolates. Of the 10 water samples that were inoculated into cetrimide agar plates, only 1 grew giving a green coloration on the plates confirming it as *Pseudomonas aeruginosa isolate.* This isolate was labeled A1 to differentiate it from other *Pseudomonas aeruginosa* isolates. From the 45 soil samples that were inoculated, 27 of them grow on the Cetrimide agar plates but only 21 grew giving a green coloration on the plates confirming them as *Pseudomonas aeruginosa*. These 19 isolates were labeled B1 to B19, C4 and C11 as a unique identity to differentiate them from other *Pseudomonas aeruginosa* isolates. None of the cetriamide agar plates inoculated with the skin swap samples had growth on them.

Morphological and Biochemical Characterization of Pseudomonas aeruginosa Isolates

Pseudomonas aeruginosa isolates showed pinkish, Gram negative rods with scattered cell arrangements under microscopy. The biochemical tests confirmed that *pseudomonas aeruginosa* isolates were positive to catalase, citrate and Voges Proskauer tests while negative to coagulase, indole and methyl red tests.

Detection of Pyocin S2 Gene in Pseudomonas aeruginosa Isolates

Primers used in this study

Primer used for the amplification pyocin S2 genes in PCR (Polymerase Chain Reaction) is seen in Table 1 with a forward primer and a reverse primer nucleotide sequence, which are short sequences of DNA used to initiate the replication process during PCR.

Table 1: Primer used in the study.

Primer type	Primer sequence	PCR product size (bp)
Forward	5'-TGCTTTGCCTCAACTGACCA-3'	550
Reverse	5'-ATCCGCAATCTGGCTTGGAA-3'	

Isolates with Presence of Pyocin S2 Pyocin Genes

Out of the 20 *Pseudomonas aeruginosa* isolates that were screened for the presence of pyocin S2 11 of the isolates were identified to possess the genes (Figure 1). These include Isolate; CL2, CL3, CL6, CL8, CL9, A1, B2, B3, BI0, B19 and C11.

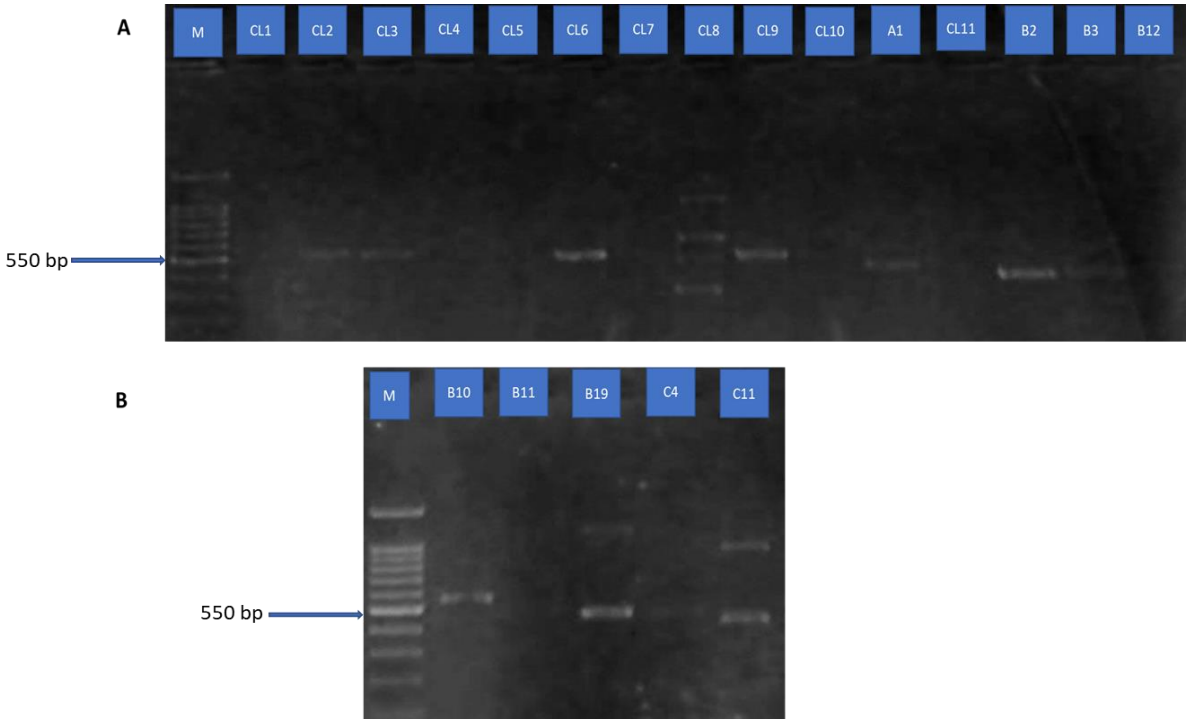

Figure 1:(a) Agarose gel electrophoresis images of the PCR products . M: Molecular ladder; and PCR products: CL1, CL2, CL3, CL4, CL5, CL6, CL7, CL8, CL9, CL10, A1, CL11, B2, B3 and B12 (b) Agarose gel electrophoresis images of the PCR products . M: Molecular ladder; and PCR products: B10, B11, B19, C4 and C11.

Antimicrobial Activity of Pyocin S2 on Target Organisms

Pyocin S2 from *Pseudomonas aeruginosa* isolate A1, B2, CL2, CL8 and C11 exhibited antimicrobial activity against *Escherichia coli* (Table 2, Figure 1) with C11 having the highest antimicrobial activity recording a mean zone of inhibition of 29.0mm while B2 has the lowest antimicrobial activity measuring a mean zone of inhibition of 17.5mm. However pyocin S2 from the aforementioned strains did not suppress the growth of *Staphylococcus aureus*, *Salmonella sp.*, and *Streptococcus pneumoniae.* Pyocin S2 from *Pseudomonas aeruginosa* isolate B3, B10, CL3, B19, CL9 and CL6 did not exhibited antimicrobial activity on *Escherichia coli*.

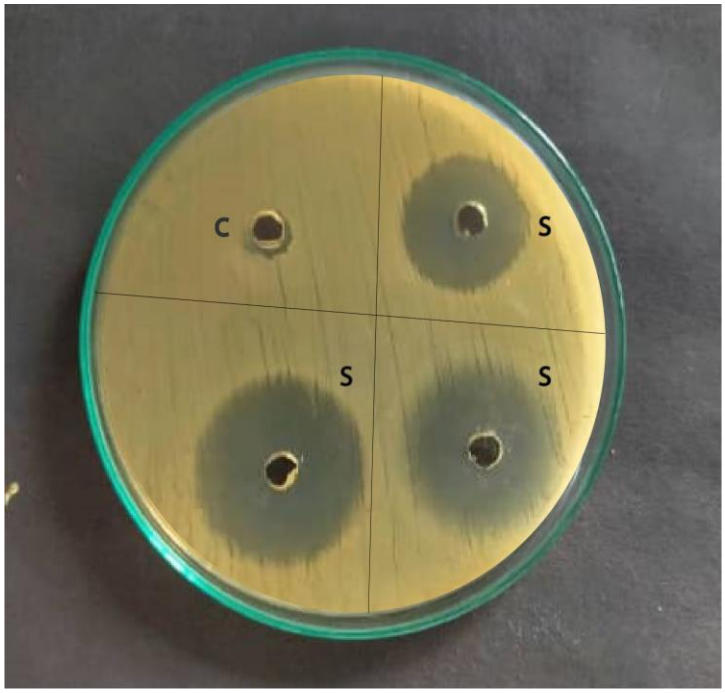

Figure 2: Typical zone of inhibition produced by bacteriocin from *Pseudomonas aeruginosa*. C: control (uninoculated broth without bacteriocin), S: replicates of cell-free supernatant from 24-hour nutrient broth culture of *Pseudomonas aeruginosa*

Table 2: Antimicrobial Activity of Pyocin S2 from various strains of *Pseudomonas aeruginosa* against *Escherichia coli.*

Pyocin S2	Zone of Inhibition (mm)
A1	23.5±3.54[c]
B2	17.5±3.54[e]
CL2	26.0±0.00[b]
CL8	21.5±0.71[d]
C11	29.0±1.00[a]
B3	0.0± 0.00[f]
B10	0.0 ± 0.00[f]
CL3	0.0 ± 0.00[f]
B19	0.0 ± 0.00[f]
CL9	0.0 ± 0.00[f]
CL6	0.0 ± 0.00 [f]

A1: pyocin S2 from *P. aeruginosa* A1, B2: pyocin S2 from *P. aeruginosa* B2, CL2: pyocin S2 from *P. aeruginosa* CL2, CL8: pyocin S2 from *P. aeruginosa* CL8, C11: pyocin S2 from *P. aeruginosa* C11, B3: pyocin S2 from *P. aeruginosa*B3, B10: pyocin S2 from *P. aeruginosa*B10, CL3: pyocin S2 from *P. aeruginosa*CL3, B19: pyocin S2 from *P. aeruginosa*B19, CL9: pyocin S2 from *P. aeruginosa* CL9, CL6: pyocin S2 from *P. aeruginosa* CL6
Values are means of 3 replications ± Standard Deviation; means that differ significantly at 95% confidence level were assigned different alphabet

Pyocin S2 from *Pseudomonas aeruginosa* isolate B3, B10, CL3, B19, CL9 and CL6 exhibited antimicrobial activity on *Staphylococcus sp* (Table 3) but not on *Staphylococcus aureus, Salmonella sp., and Streptococcus pneumoniae*. Pyocin S2 from *Pseudomonas aeruginosa* isolate CL3 exhibited the highest inhibitory activity as shown by a mean zone of inhibition of 28.5mm while Pyocin S2 from *Pseudomonas aeruginosa* isolate B10 had the lowest inhibitory activity with mean zone of inhibition of 18.5mm. Pyocin S2 from *Pseudomonas aeruginosa* isolate A1, B2, CL2, CL8 and C11 did not suppress the growth of *Staphylococcus sp*.

Table 3: Antimicrobial Activity of Pyocin S2 from various strains of *Pseudomonas aeruginosa* against *Staphylococcus sp.*

Pyocin S2	Zone of Inhibition(mm)
A1	0.0 ± 0.00[f]
B2	0.0 ± 0.00[f]
CL2	0.0 ± 0.00[f]
CL8	0.0 ± 0.00[f]
C11	0.0 ± 0.00[f]
B3	21.0±1.41[d]
B10	18.5±0.71[e]
CL3	28.5±0.71[a]
B19	23.7±2.31[c]
CL9	24.5±6.36[b]
CL6	23.5±4.95[c]

A1: pyocin S2 from *P. aeruginosa* A1, B2: pyocin S2 from *P. aeruginosa* B2, CL2: pyocin S2 from *P. aeruginosa* CL2, CL8: pyocin S2 from *P. aeruginosa* CL8, C11: pyocin S2 from *P. aeruginosa* C11, B3: pyocin S2 from *P. aeruginosa*B3, B10: pyocin S2 from *P. aeruginosa*B10, CL3: pyocin S2 from *P. aeruginosa*CL3, B19: pyocin S2 from *P. aeruginosa*B19, CL9: pyocin S2 from *P. aeruginosa* CL9, CL6: pyocin S2 from *P. aeruginosa* CL6
Values are means of 3 replications ± Standard Deviation; means that differ significantly at 95% confidence level were assigned different alphabet

Pyocin S2 from *Pseudomonas aeruginosa* isolate C11 also exhibited antimicrobial activity against *Vibrio cholerae* (Table 4) with a mean zone of inhibition = 30.7mm, but Pyocin S2 from *Pseudomonas aeruginosa* isolate CL2, CL8, CL9, CL6, A1, B2, B3, B10, CL3, B19 did not inhibit the pathogen.

Table 4: Antimicrobial Activity of Pyocin S2 from various strains of *Pseudomonas aeruginosa* against *Vibrio cholerae*.

Pyocin S2	Zone of Inhibition(mm)
A1	0.0 ± 0.00^{b}
B2	0.0 ± 0.00^{b}
CL2	0.0 ± 0.00^{b}
CL8	0.0 ± 0.00^{b}
C11	30.7 ± 1.15^{a}
B3	0.0 ± 0.00^{b}
B10	0.0 ± 0.00^{b}
CL3	0.0 ± 0.00^{b}
B19	0.0 ± 0.00^{b}
CL9	0.0 ± 0.00^{b}
CL6	0.0 ± 0.00^{b}

A1: pyocin S2 from *P. aeruginosa* A1, B2: pyocin S2 from *P. aeruginosa* B2, CL2: pyocin S2 from *P. aeruginosa* CL2, CL8: pyocin S2 from *P. aeruginosa* CL8, C11: pyocin S2 from *P. aeruginosa* C11, B3: pyocin S2 from *P. aeruginosa*B3, B10: pyocin S2 from *P. aeruginosa*B10, CL3: pyocin S2 from *P. aeruginosa*CL3, B19: pyocin S2 from *P. aeruginosa*B19, CL9: pyocin S2 from *P. aeruginosa* CL9, CL6: pyocin S2 from *P. aeruginosa* CL6

Values are means of 3 replications ± Standard Deviation; means that differ significantly at 95% confidence level were assigned different alphabet

Discussion

Pseudomonas aeruginosa was isolated from soil samples, water and clinical (human wound) samples. This shows *Pseudomonas aeruginosa* is a versatile bacterium with the ability to thrive in a wide range of habitats, making it a significant concern in both natural and man-made environments (Peix *et al.*, 2009; Diggle and Whiteley, 2020; Doring *et al.*, 2010; Haas and Keel, 2003; Krell and Matilla, 2024; Stover *et al.*, 2000). The presence of *Pseudomonas aeruginosa* isolates in clinical samples can be attributed to the ability of the bacteria to adapt and develop resistance to several antibiotics (Moradali *et al.*, 2017).

The presence of pyoverdine in the isolates of the *Pseudomonas aeruginosa* confirmed the identity of these isolates by producing a distinct florescent yellow pigment growth on cetrimide agar plates (Yilmaz, 2017). Further confirmation using biochemical showed that the *pseudomonas aeruginosa* strains produce catalase and citrate utilization enzymes thereby giving a positive result to catalase and citrate utilization tests respectively. The *Pseudomonas aeruginosa* strains gave a negative test results for indole, methyl red and Voges Proskauer test due to the absence of tryptophanase enzyme production which hydrolizes tryptophan to produce indole, inability of the bacteria to convert glucose in the medium to acids and inability to metabolise pyruvate into acetylmethylcarbinol for each test respectively (Shoaib *et al*., 2020).

The detection of pyocin S2 gene in 11 of the *Pseudomonas aeruginosa* isolates signifies that indigenous strains contain pyocin S2 gene. All the isolates found to contain the pyocin S2 gene were also observed to produce pyocin S2. Pyocin S2 in this study suppressed the growth of *Escherichia coli*, *Staphylococcus sp.,* and *Vibrio cholerae.* Although the antimicrobial activity of S-type pyocin against *Escherichia coli* has not been well documented (Suleiman *et al.*, 2024) engineered R-type pyocin AVR2-V10 has been specifically targeted to kill *Escherichia coli* 0157:H7, a major food-borne pathogen (Scholl *et al.,* 2009). The findings of this study which demonstrates the inhibition of growth of Staphylococcus sp. by pyocin S2 is in line with a study that found that pyocin S2 exhibit the ability to inhibit the growth of *Staphylococcus aureus*, *Staphylococcus epidermidis*, and other *Staphylococcus sp.* (Charkhain *et al*., 2024). Therefore, Pyocin S2 can be developed as a therapeutic agent to fight infections caused by *Staphylococus sp.* The prominent antimicrobial activity of pyocin S2 from *Pseudomonas aeruginosa* isolate C11 on *Vibrio cholerae* from this research work shows that pyocin S2 suppresses the growth of *Vibrio cholerae.* Hence it high potential to act as a strong therapeutic agent against *Vibrio cholerae* infections. This is the first report of the antimicrobial activity of pyocin S2 against *Vibrio cholerae*.

CONCLUSION

Out of 20 *Pseudomonas aeruginosa* subjected to PCR, 11 contained pyocin S2 gene. Pyocin S2 from *Pseudomonas aeruginosa* isolate A1, B2, CL2, CL8 and C11 produced zone of inhibition (ZOI) when tested against *Escherichia coli*. However, pyocin S2 from *Pseudomonas aeruginosa* B3, B10, CL3, B19, CL9 and CL6 did not inhibit *Escherichia coli* but suppressed the growth of *Staphylococcus sp*. Also, Pyocin S2 from *Pseudomonas aeruginosa* C11 suppressed the growth of *Vibrio cholerae*, but not the other strains. The therapeutic potentials of pyocin S2 can be futher explored to overcome diseases caused by *Escherichia coli*, *Staphylococcus sp*, and *Vibrio cholerae*.

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