



Antimicrobial Efficacy of Bacteriocin from *Leuconostoc mesenteroides* subsp. *cremoris* Against *Pseudomonas aeruginosa*

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ABSTRACT

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Leuconostoc mesenteroides subsp. *cremoris*, which were isolated from raw milk and some fruits and vegetables, specify fermented foods such as sauerkraut, milk products, and some pickles. It was obtained and examined for its antagonistic activity against spoiled isolates and its antibacterial activity against *Xanthomonas* sp. and *Erwinia* sp., and spoilage mold. Antibacterial and antibiofilm effects of bacteriocin were evaluated against *Pseudomonas aeruginosa* isolated from wounds. Bacteriocin was first extracted from *Leuconostoc mesenteroides* spp. Then the bacteriocin was purified and characterized using Fourier Transform Infrared Spectroscopy (FTIR) and an amino acid analyzer. The FTIR of purified bacteriocin revealed that the functional groups in bacteriocin were NH₂, CH, C=C, C-O-C, carbonyl group, and the absence of the OH group. The analysis of amino acids showed that the purified bacteriocin contained 12 types of amino acids. The activity of antibacterial purified bacteriocin was investigated against *P. aeruginosa* isolates using the co-culture method. The purified bacteriocin showed an inhibitory effect against *P. aeruginosa* isolates, with a reduction of growth reaching about 100%, according to the bacterial colonies. In addition, the effects of bacteriocin on cell permeability, pyocyanin production, and biofilm were investigated, and the results showed that purified bacteriocin caused an increase in cell permeability and a reduction of pyocyanin production. Also, the purified bacteriocin showed a clear antibiofilm effect against *P. aeruginosa* isolates in both the microtiter plate method and scanning electron microscope (SEM) method, with the highest biofilm degradation being 60.14%. The bacteriocin purified from *Leuconostoc mesenteroides* subsp. *Cremoris* had an inhibitory effect against the growth of *P. aeruginosa* and its biofilm, pyocyanin production, and cell permeability.

1. INTRODUCTION

Leuconostoc mesenteroides subsp. *cremoris* produces a variety of organic acids and some chemical elements that are antibacterial and known as bacteriocins (such as carnosyn and leuconosin) [1, 2]. Bacteriocins are proteins produced by various bacterial strains and are composed of ribosomes (anti-infection) [3], thus bacteriocins are considered one of the ways to treat difficult infections [4].

Bacteriocins can be classified into four classes (I, II, III, IV), which include: Class I Bacteriocins include small, heat-resistant and proteolytic peptides, this class can be divided into types A and B. Class II bacteriocins, it consists of small peptides that are resistant to temperature and acidity, Class III bacteriocins, which are proteins that are highly thermostable (~10 kDa). Class IV bacteriocins consist of complex proteins that bind to fats or carbohydrates [5, 6]. And the class of bacteriocin from *L. mesenteroides*, which produces bacteriocin in high quantity, corresponds to a typical feature of the class IIa pediocin-like bacteriocins [7]. Despite this, a

study on LAB bacteria, which includes their effect against pathogenic bacteria, such as multi-drug-resistant bacteria, viruses, and yeasts [8]. As noted by LAB, they may be safe and are often used in the preservation of dairy products, may provide new approaches through developing treatments and improving food safety, as is the case with antibiotic-resistant bacteria such as *Pseudomonas aeruginosa* [9], in an attempt to solve the problem of antibiotic resistance of these bacteria.

Pseudomonas aeruginosa is one of the most important species of Gram-negative bacteria, and one of the most important pathogens of major diseases such as cystic fibrosis, burn infections, and urinary tract infections [10].

Pseudomonas aeruginosa is a common causative agent of wound infections, and its complete eradication often remains challenging due to its ability to inflict extensive tissue damage, necessitating the use of potent antibacterial agents [11]. This pathogen demonstrates remarkable adaptability within hostile host environments through the secretion of diverse virulence factors that facilitate successful colonization and disease progression [12]. Among its major virulence determinants are

lipopolysaccharide (LPS), the phenazine pigment pyocyanin (PCN), and its capacity for robust biofilm formation [13, 14]. The objective of this study was to investigate the effects of a purified and characterized bacteriocin from *Leuconostoc mesenteroides* subsp. *cremoris* on the growth and certain virulence factors of *Pseudomonas aeruginosa*.

2. MATERIALS AND METHODS

2.1 Microorganism

Nineteen isolates of *P. aeruginosa* were isolated from wound infections collected from the Department of Biology, College of Science, Mustansiriyah University. These isolates were identified throughout cultural, microscopical, and biochemical tests and the Vitek2 system.

2.2 Antibiotic susceptibility test

To create the bacterial suspension, four to five colonies of all isolates of bacteria were selected from the initial starter culture, and then put into a plain tube with four milliliters of physiological saline. After that, the density of bacterial growth was compared with 0.5 standard MacFarland (1.5×10^8 CFU/ml). A portion of the bacterial suspension was gently and evenly placed over Mueller-Hinton agar medium using a sterile cotton swab, and it was then allowed to dry completely at room temperature. Using sterile forceps, the antibiotic discs were firmly pressed onto the agar to ensure that they contacted it. The plates were then turned over and left to incubate for a full day at 37°C. Zones of inhibition that formed around the discs were measured in millimeters (mm) using a metric ruler, as per the Clinical Laboratory Standards Institute. Based on these measurements, the isolate was classified as either susceptible or resistant to a specific antibiotic.

2.3 Detection of biofilm formation in *Pseudomonas aeruginosa* isolates

Bacterial isolates were tested for their ability to form biofilm. 96 flat-bottomed microtiter plates were filled with 180 µl of brain heart infusion broth with 2% sucrose, and 20 µl of the overnight bacterial culture equivalent to 0.5 McFarland standard was added as an inoculant. 200µl of brain heart infusion broth with 2% sucrose was placed in the control wells. The covered microtiter plate was sealed with parafilm and incubated at 37°C for 24 hours. To remove any unattached bacterial cells, wash the wells three times with PBS (pH 7.2), let them dry for 15 minutes at room temperature, and then add 200µl of crystal violet (0.1%) for 20 minutes. Following the withdrawal of the crystal violet solution, the wells underwent three rounds of washing with PBS (pH 7.2) to eliminate any remaining dye. They were then left to dry at room temperature and twice extracted using 200µl of 95% ethanol. The absorbance of each well was then measured using an ELISA reader at 630 nm. The biofilm formation of each isolate was categorised into the following categories based on the absorbance values: $OD \leq OD_c$ (Non), $OD_c < OD \leq 2 OD_c$ (Weak), $2 OD_c < OD \leq 4 OD_c$ (Moderate), and $4 OD_c < OD$ (High).

2.4 *Leuconostoc mesenteroides* subsp. *cremoris*

Leuconostoc mesenteroides subsp. *cremoris* was collected

from the Department of Biology, College of Science, Mustansiriyah University. This isolate was identified through cultural, microscopical, and biochemical tests and the Vitek2 system according to studies [15, 16].

2.5 Extraction of bacteriocin from *L. mesenteroides* subsp. *cremoris*

MRS broth was used as an inoculated for the bacterial isolate and allowed to incubate for 24 hours at 30°C. Centrifugation at 10,000 rpm for 15 minutes was done to obtain the supernatant was filtered using a Millipore filter 0.02 µm, then neutralised to pH 6.2 in order to counteract the effects of organic acid, using 1N NaOH [16]. Dialysis bags and polyethylene glycol 20000 were used to concentrate the crude bacteriocin [17].

2.6 Purification of Bacteriocin Produced by *L. mesenteroides* subsp. *cremoris* using Sepacryl S-200 gel

First, the gel (provided by the Swedish company Pharmacia) is washed using a phosphate buffer solution pH 6.2, after which an emptying step is performed. The gel is then filled using a column to give a final size of 1.5×60 cm in order to avoid the formation of air bubbles. To ensure the required pressure of the separation material, the column is balanced for 24 hours, after which the required flow speed is calculated and the column position is adjusted, until the concentrated supernatant is added to the top of the column so that the separated parts are collected at a flow rate 18 ml/h of 3 ml/part, then the O.D of separated parts are read using Spectrophotometer at 280 nm. Protein concentration was quantitatively estimated for the separated peaks [18].

2.7 Characterization of bacteriocin

2.7.1 Fourier transform infrared spectroscopy (FTIR)

The sample was examined with an FTIR spectroscopy device. The diagnosis was made in the Chemistry Department, College of Sciences, Mustansiriyah University, Baghdad, Iraq. This device measures the amount of reflected infrared radiation at wavelengths $400\text{--}4000\text{ cm}^{-1}$. The results were read using a graph with the x-axis representing the wavelength cm^{-1} , while the y-axis represents the wavelength Transmittance %. The resolution was set at 4 cm^{-1} with 32 scans.

2.7.2 Amino acid analysis

The sample was examined at the Ministry of Science and Technology, Baghdad, Iraq. The amino acid components of bacteriocin were identified.

2.8 Effect of purified bacteriocin on *P. aeruginosa*

2.8.1 Antibacterial activity of purified bacteriocin against *P. aeruginosa* isolates

The effect of bacteriocin purified from *Leuconostoc mesenteroides* subsp. *cremoris* against *P. aeruginosa* was studied in triplicate. Initially, *P. aeruginosa* isolates were grown in nutrient broth with bacteriocin (1:1) while the control medium contained only nutrient broth. The co-cultures and control were then incubated at 37°C for 24 hours. After incubation, 1 ml of each culture was serially diluted, and 0.1 ml was taken from each dilution and cultured on nutrient agar plates. The plates were incubated at a 37°C for 24-48 hours

[16]. The colonies were counted, and the inhibition activity was evaluated and calculated percent reduction of bacteria using the following equation described as [19]:

$$R (\%) = [A - B] / A \times 100$$

where, R = the reduction rate, A = the number of bacterial colonies from the control medium, and B = the number of bacterial colonies from the treatment with purified bacteriocin.

2.8.2 Effect of purified bacteriocin on *P. aeruginosa* cell permeability

A centrifuge was used at 6000 rpm for 15 minutes to extract 10 ml of fresh culture broth of *P. aeruginosa* bacteria. The cells were then washed twice with sterile 5 mM potassium phosphate buffer (pH 6.5) and resuspended in 10 ml of the same buffer. Bacteria were added to the washed cells at a ratio of 0.1:1.0. Cells were collected by centrifugation at 6000 rpm for 15 min after 1 h of incubation at 37°C, and the supernatant was filtered through a 0.20 µm filter membrane. Optical density measurement at 260 nm was used to calculate DNA concentration. Both cell suspension in 5 mM potassium phosphate buffer (pH 6.5) without bacteriocin and the same buffer containing bacteriocin without the bacterial cell were used as control [20, 21]. The experiment was done in triplicate.

2.8.3 Effect of purified bacteriocin on pyocyanin production by *P. aeruginosa*

The effect of bacteriocin against pyocyanin production by *P. aeruginosa* was studied in triplicate which the *P. aeruginosa* isolates were diluted 1:100 in nutrient broth after growing in the nutrient medium and then treated with purified bacteriocin (1:1). After growth for 16 hours, the culture supernatants were collected in a centrifuge at 6000 rpm for 15 minutes, then transferred to a cuvette and photographed [22].

2.8.4 Degradation of *P. aeruginosa* biofilm by purified bacteriocin from *Leuconostoc mesenteroides* subsp. *cremoris*

The degradation of biofilm by purified bacteriocin was tested in a microtiter plate using an appropriate medium, brain heart infusion supplemented by 2% sucrose. Depending on the procedure mentioned by study [23], the plate was inoculated with bacterial suspension compared to 0.5 MacFarland. The final volume of medium in each well was 200 µl, each well contained 180 µl of brain heart infusion broth with 2% sucrose and 20 µl of bacterial suspension. The control negative contained only 200 µl Brain Heart Infusion with 2% sucrose, while the control positive contained 180 µl of Brain Heart Infusion broth with 2% sucrose and 20 µl of bacterial suspension. After incubation at 37°C for 72h the broth was removed then added 200 ml suspension purified bacteriocin, incubated for 24h at 37°C, then purified bacteriocin was removed from wells and washed with Phosphate–buffer saline (pH 7.2) to remove unattached stain and left to dry at room temperature for 15 min, finally, 200 µl of 95% ethanol was added to each well and optical density was read at 630 nm by Eliza reader. The degradation of biofilm percentage was calculated as follows:

$$\text{Degradation of biofilm\%} = \frac{\text{O.D. Control} - \text{O.D. Treatment}}{\text{O.D. Control}} \times 100$$

2.8.5 Detection of the effect of bacteriocin on *P. aeruginosa* biofilm using scanning electron microscopy

A scanning electron microscope was used to examine the effect of bacteriocin on the biofilms of *P. aeruginosa*, where *P. aeruginosa* isolates were grown on coverslips coated with poly(L-lysine). After adding bacteriocin at a concentration of 1.94 µg/ml, they were then incubated for 24 hours. Then the samples were fixed using Karnofsky's fixative for 2 hours at 4°C and typically rinsed with phosphate buffer, washed, and dried with a graded ethanol series and finally with tert-butyl alcohol. Next, cover slides were dipped in tert-butyl alcohol and stored at -20°C, followed by freeze-drying and platinum coating. Samples were observed using a Zeiss EVO 40 instrument (Ukraine) [24].

The cell permeability of *P. aeruginosa* was increased after purified bacteriocin treatment, the O.D was recorded (0.197, 0.383, 0.448, 0.714, 0.385) after treatment with bacteriocin, compared with control (0.010, 0.049, 0.039, 0.139, 0.054) for each isolate (4, 5, 7, 18, 19), significant difference P-value < 0.05 was recorded between O.D. before and after bacteriocin treatment as shown in Table 1.

Statistical analysis: Microsoft Excel was used for data entry. The data were analysed using SPSS-27 (Statistical Package for Social Sciences version 27). Shapiro-Wilk and Kolmogorov-Smirnov were employed to assess the normality of distribution. The paired t-test was used to evaluate the difference between O.D. before and after bacteriocin treatment. P-value ≤ 0.05 was considered significant.

Table 1. Detection of amino acids in bacteriocin purified from *Leuconostoc mesenteroides* subsp. *cremoris*

	Type of Amino Acid	Reten. Time (min)	Amount (mg)
1.	Aspartic acid	4.388	0.003
2.	Glutamic acid	7.852	75.492
3.	Serine	8.576	12.963
4.	Glycine	8.836	46.085
5.	Alanine	9.920	65.974
6.	Cystine	11.432	5.684
7.	Valine	12.096	68.444
8.	Methionine	12.420	44.255
9.	Isoleucine	12.936	23.218
10.	Leucine	13.340	70.067
11.	Phenylalanine	13.804	61.313
12.	Lysine	14.084	38.973

3. RESULTS AND DISCUSSION

3.1 Antibiotic susceptibility test

All isolates of *P. aeruginosa* were detected by antibiotic susceptibility test using the disc diffusion test, which involved nine different antibiotic classes: Cefalexin, Rifampin, Cefixime, Ciprofloxacin, Amikacin, Doxycycline, Cefotaxime, and Gentamicin. The results were interpreted according to the recommendation of CLSI (2023)

As illustrated in Figure 1, all isolates were resistant to antibiotics (cephalexin, rifampin, cefixime, doxycycline, cefotaxime) in the rate of 100% and 26, 78, 15% were resistant to antibiotics (ciprofloxacin, amikacin, and gentamicin), while the percentage of isolates was the average rate of isolates was 47% and 84% for both ciprofloxacin and gentamicin.

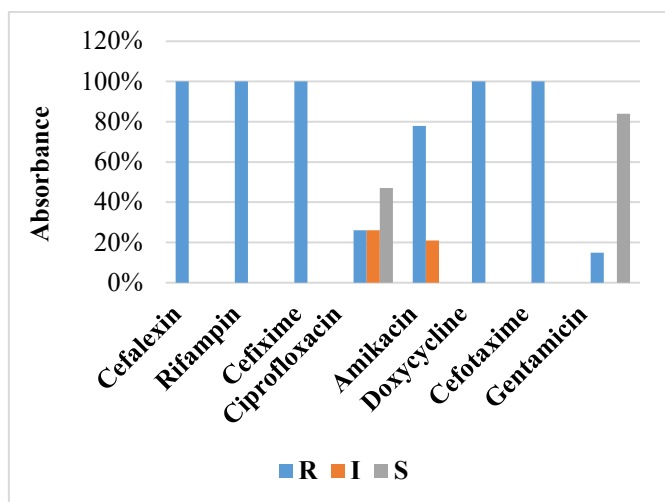


Figure 1. Antibiotic susceptibility test of antibiotics (*P. aeruginosa*)

Note: R: Resistance, I: Intermediate, S: Sensitive versus their activity

P. aeruginosa produced the highest rates of resistance to cefepime (56.67%), gentamicin (57.97%), followed by fluoroquinolones (55.11%) and carbapenems (55.02%), while the highest susceptibility rate was 97.41% to colistin [14].

3.2 Biofilm formation of *P. aeruginosa* isolates

The results in Table 2 show variation in biofilm formation among *P. aeruginosa* isolates. Out of 19 isolates, five were unable to form biofilms, three had moderate biofilm-forming ability, and eleven had weak biofilm-forming ability.

Table 2. Detection of biofilm formation in *Pseudomonas aeruginosa* isolates

Bacterial Isolates	Biofilm Formation		
	Number of Biofilm Bacterial Isolates (Number of Total Bacterial Isolates)		
	Non	Moderate	Weak
<i>P. aeruginosa</i>	5 (19)	3 (19)	11 (19)

In addition, bacteria have the ability to influence the pH of the environment, which helps promote biofilm growth, as has been found in the formation of biofilms on urinary catheters by *Klebsiella* and *Pseudomonas* through increased urine alkalinity. On the other hand, flagellar bacteria can experience hydrodynamic stress and thus have an advantage in biofilm formation. In the case of biological surfaces, the hydrophobicity of interacting cell membranes is essential for the attachment of bacteria to the host surface, which occurs primarily through the interaction of bacterial proteins with extracellular proteins or carbohydrate fragments on the surface of cells/tissues [15].

3.3 Purification of bacteriocin using gel filtration

Sephacryl S-200 was used to purify bacteriocin by adding the sample obtained after concentration with polyethylene glycol 20000 to a gel filtration column with dimensions of 1.5 × 60 cm to purify bacteriocins from *Leuconostoc mesenteroides* subsp. *cremoris*. The accuracy of the process of purifying the separated proteins was verified using a polyacrylamide gel, in addition to the presence of auxiliary

factors. Electrophoresis was also performed, where the results showed the appearance of a single band of bacteriocin, with the protein ranging from 0.596 to 1.941 mg/ml.

3.4 Characterization of bacteriocin

3.4.1 Transform Infrared Spectroscopy (FTIR)

The FTIR results of purified bacteriocin is showed in Figure 2 that the bands in the region 3343 cm⁻¹ was due to NH₂ while the bands in the region 3285 and 1621 cm⁻¹ were due to (C=C) stretching, the band region 2168 cm⁻¹ was due to (C-H) while the band region 1499 cm⁻¹ was due to (CH deformation for CH₃), the band region 1398 cm⁻¹ was due to (C-O-C) while the band region 1262 cm⁻¹ was due to indicate disappearance of OH and the bands 1077 and 1033 cm⁻¹ were due to Carbonyl group.

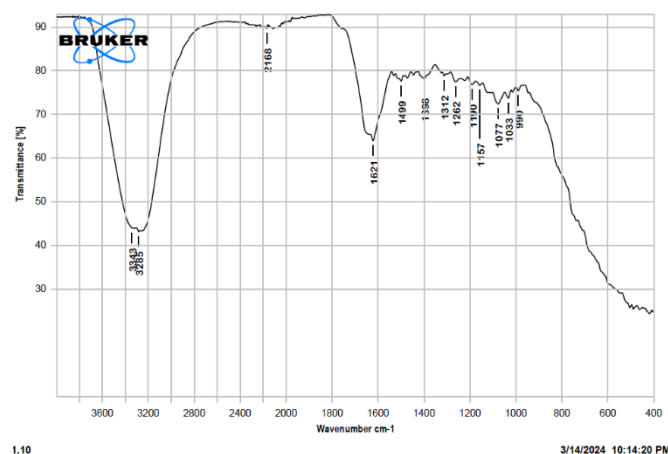


Figure 2. FTIR of bacteriocin purified from *Leuconostoc mesenteroides* subsp. *cremoris*

For *P. acidilactici* B1153 bacteriocin, the FTIR showed a drop in spectra in the 1,650–1,055.8 cm⁻¹ region, suggesting deformations in aliphatic, including phosphate bond, carbonyl group stretching, and C–O–C deformations; they may include polysaccharides, glycolipids, and phosphodiester. Additionally, the 3,000–3,500 cm⁻¹ region, which is consistent with NH₂ stretching [16].

3.4.2 Amino acid analysis of bacteriocin from *L. mesenteroides* subsp. *cremoris*

Bacteriocin from *Leuconostoc mesenteroides* subsp. *cremoris* was formed from 12 types of amino acids, including Aspartic acid, glutamic acid, Serine, Glycine, Alanine, Cystine, Valine, Methionine, Isoleucine, Leucine, Phenylalanine, and Lysine (Table 1).

Some types of bacteriocins are peptides consisting of only 13 to 37 amino acids. The unusual amino acids found in some small bacteria are the result of post-translational changes to the more common amino acids [17]. Additionally, phenylalanine and glutamic acid were found in large concentrations in the peptide, while threonine, aspartic acid, serine, glycine, proline, isoleucine, and leucine were found in residues (Pro, Gly, Ala, Val, Ile, Leu, Phe) were estimated at 0.92% at a rate of (0.92%, 0.83% and 0.92%) for each of *L. brevis*, *L. plantarum* and *L. fermentum*. Basic amino acid contents (Arg + Lys + His) are 0.23% for all samples tested, while Acidic amino acid residues (Asp + Glu) are 0.74% for all samples tested [3].

The circulating bacteria are composed of 59% hydrophobic amino acid residues (Ile, Ala, Val, Trp, Leu, Phe, and Pro) and uncharged hydrophilic amino acid residues (32%) (Gly, Thr, Ser, Gln). In addition to a high percentage of basic amino acids (Lys, Arg, and His) compared to the presence of acidic amino acids (Asp), which confirms the strength of the basic protein character [18]. Additionally, Leucocyclicin Q, a unique cyclic bacteriocin produced from *L. mesenteroides* TK41401, was also discovered. This form of bacteriocin was discovered in Japanese pickles. Lactocyclicin Q, a cyclic bacteriocin generated by *Lactococcus* sp. strain QU 12, and leucocyclicin Q, which contains 61 amino acid residues and a cyclic structure with N and C termini attached to one another, are quite similar. Leucocyclicin Q has good stability against many bacterial proteases and a distinct antibacterial spectrum [19].

3.4.3 Effect of purified bacteriocin against *P. aeruginosa*

Figure 3 shows the antibacterial efficacy of purified bacteriocin from *L. mesenteroides* subsp. *Cremoris* against *Pseudomonas aeruginosa*. The antibacterial activity of purified bacteriocin against *P. aeruginosa* was confirmed, showing growth inhibition with reduction ratios of 100%, 100%, 97%, 99%, and 100% for isolates 4, 5, 7, 18, and 19, respectively.

The bacteriocin has the ability to function as an antioxidant and demonstrates antagonistic activity against *P. aeruginosa* [20]. Ghapanvari et al. [4] investigated the effect of nisin on *Pseudomonas aeruginosa* isolates and reported resistance, which was attributed to mutations within the bacterial cell as well as to the concentration of nisin applied.

The result of the study [21] showed that all concentrations of bacteriocins have the ability to inhibit *P. aeruginosa* isolates, and that the antibacterial effect of the bacteriocin is increased by increasing the concentration of bacteriocins. And results showed that bacteriocin from *L. rhamnosus* has antibacterial activity against *P. aeruginosa* through decreased lipopolysaccharide synthesis, and reached zero after five hours [22]. And the effect of proteolytic enzymes (trypsin and pepsin) was also diagnosed, as the activity of bacteriocins produced by *Leuconostoc mesenteroides* showed its effectiveness against all microorganisms [17]. Furthermore, mecentricin and sacacin genes were carried by *L. mesenteroides* and *L. Saki*, as inhibition was observed against some types, such as *Enterococcus faecalis*, *Shigella dysenteriae*, and *Escherichia coli* O157:H7 [23].

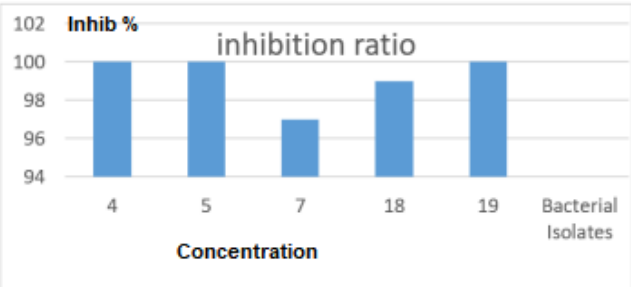


Figure 3. Antibacterial efficacy of purified bacteriocin derived from *Leuconostoc mesenteroides* subsp. *cremoris* against *Pseudomonas aeruginosa*

3.4.4 Effect of purified bacteriocin on *P. aeruginosa* cell permeability

The cell permeability of *P. aeruginosa* was increased after purified bacteriocin treatment, the O.D. was recorded (0.197,

0.383, 0.448, 0.714, 0.385) after treatment with bacteriocin, compared with control (0.010, 0.049, 0.039, 0.139, 0.054) for each isolate (4, 5, 7, 18, 19), significant difference P-value < 0.05 was recorded between O.D. before and after bacteriocin treatment as shown in Table 3.

Table 3. Effect of purified bacteriocin from *Leuconostoc mesenteroides* subsp. *cremoris* on *P. aeruginosa* cell permeability

Bacterial Isolates	O.D. (260nm) without Bacteriocin Treatment	O.D. (260nm) after Bacteriocin Treatment
<i>P. aeruginosa</i> (4)	0.01	0.197
<i>P. aeruginosa</i> (5)	0.049	0.383
<i>P. aeruginosa</i> (7)	0.039	0.448
<i>P. aeruginosa</i> (18)	0.139	0.714
<i>P. aeruginosa</i> (19)	0.054	0.385
Mean ± SD	0.058 ± 0.048	0.425 ± 0.186
t	d.f.	*P-value
5.813	4	0.004

Note: *P-value < 0.05 was considered significant

The bacteria may have holes in the cell membrane on their surface, where the cell membrane changes, leading to cell death in addition to a decrease in the biomass of *P. aeruginosa* PAO1 after bacteriocin was treatment, which leads to cell death, the combination of both bacteriocins with exopolysaccharides has a more effective effect, and their combination together leads to a significant decrease in the ability to form a membrane [24]. A reduction in the composition of the smooth cell membrane, as an increase in the formation of pores and leakage in the entry of cell components, was clear evidence of a defect in the cell membrane. The results also indicated that bacteriocin causes changes in the composition of the cell membrane, which leads to cell death [20]. It was also observed in the use of nisin A, which is produced by *Lactococcus* bacteria. This antibiotic binds to lipid II, which is associated with the formation of the cell wall, and this binding prevents the formation of cells well. This leads to increased pore formation by nisin molecules, which quickly kill cells [25]. As pointed out by study [26], bacteriocin absorption may have chemical effects on the cell, including interference with the mitotic process of 16S ribosomal RNA, degradation of cellular DNA, and inhibition of the formation of the peptidoglycan layer. It was also observed when Nisin (NS) and Citric Acid (CA) were added alone or together against both *S. aureus* and *L. monocytogenes* Cell components are released by measuring absorbance at 260 nm, where the release of cell components (including macromolecules such as DNA and RNA and small ions such as K⁺ and PO₄) with strong UV absorption at 260 nm is an indicator of membrane damage [27]. The membrane rupture mechanism was also observed. It consists of two stages: (binding the peptide to the surface of the outer layer, and as a result of the surface tension of the peptide, the membrane ruptures). The explanation is that the continuous development of the pores leads to the rupture of the membrane through the process of stretching the membrane surfactants. Also, the hydrophobic interactions between the peptide layer and the lipids, as well as the assembly of cationic components, are

major components in this process. Amino acids have been shown to play a role in improving the membrane-damaging ability of bacteriocins [28] from the above results, it can be concluded that there is a relationship between 100% inhibition of bacterial growth and increased membrane permeability when using purified bacteriocin, as most of the mechanisms of antimicrobial action of bacteriocins is disruption of cell membrane, binding to specific proteins receptor on the bacterial cell membrane and forming pores that increase cell membrane permeability, finally causing cell death [29].

3.4.5 Effect of purified bacteriocin on pyocyanin production by *P. aeruginosa*

The results showed the effect of purified bacteriocin by reducing pyocyanin production compared to the control in *P. aeruginosa* (Figure 4). Pyocyanin production in *P. aeruginosa* isolates was clearly reduced compared to the pyocyanin production before the addition of *L. plantarum* [22].

Pyocyanin is a blue-green phenazine pigment produced in large quantities by active cultures of *P. aeruginosa* [29]. One of the most important factors responsible for the appearance of a number of virulence factors in *P. aeruginosa* bacteria is quorum sensing (QS), and these factors include pyocyanins, proteases, and rhamnolipids. and twitching motility. The effect of bacteriocin in reducing the virulence factors of *P. aeruginosa* bacteria, compared to the control culture of *P. aeruginosa*, such as the production of pyocyanin, decreased protease production, and rhamnolipid production is reduced and it was found to decrease the twitching motility [30].

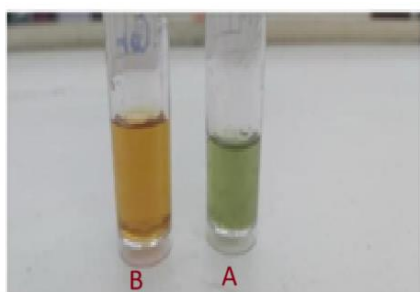


Figure 4. Effect of bacteriocin from *Leuconostoc mesenteroides* subsp. *cremoris* on pyocyanin production by *P. aeruginosa*

A: control (without bacteriocin); B: Treatment with bacteriocin

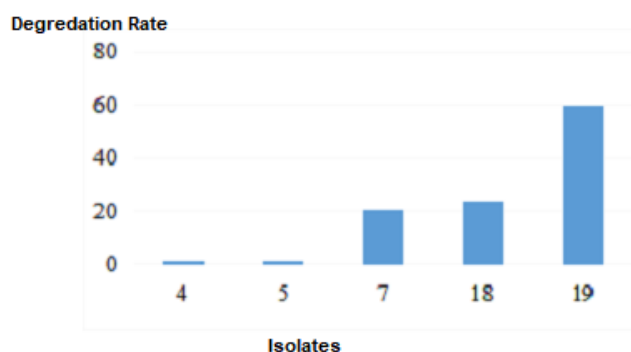


Figure 5. Degradation of *P. aeruginosa* biofilm by purified bacteriocin from *Leuconostoc mesenteroides* subsp. *cremoris*

3.4.6 Degradation of *P. aeruginosa* biofilm by purified bacteriocin from *L. mesenteroides* subsp. *cremoris*

Results showed that purified bacteriocin could degrade

biofilms formed by five antibiotic-resistant *P. aeruginosa* isolates with moderate biofilm-forming ability. The degradation rates were 1.28%, 1.27%, 20.26%, 23.52%, and 60.14% for isolates 4, 5, 7, 18, and 19, respectively (Figure 5).

The study of Lee et al. [30] showed that bacteriocin from *Pediococcus acidilactici* affects biofilm formation at a rate of (66.41, 45.77, 21.73) % at 0.5, 1, and 2 mg/ml in *P. aeruginosa*. They also observed that bacteriocin reduces biofilm formation on the surface of Stainless-steel measurement by scanning electron microscope and microtiter plate method. The effect of sub-inhibitory levels of colistin and nisin ($1/5 \times \text{MIC}$ and $1/4 \times \text{MIC}$ for respectively) can effectively prevent biofilm formation through total inhibition of growth [31]. And bacteriocin from *L. fermentum* showed antibiofilm activity against *P. aeruginosa* [32].

The effect of fifty-seven *lactobacilli* was examined in order to reduce the formation. The results showed that the presence of these strains with *P. aeruginosa* resulted in a 0-64% reduction in biofilm formation in total biofilm mass compared to *P. aeruginosa* strains alone [33]. But the study [34] showed that nisin destroys biofilm for a period ranging from 24 to 48 hours, as after 24 hours have passed, the bacteria have adapted to the conditions and biofilm formation increased, but only after 48 hours biofilm degradation occurred, and this indicates that nisin inhibition is dependent on time and rate biofilm formation. Treating *E. Coli* O157:H7 with nisin and phytic acid (PA) together showed positive synergistic bactericidal action by breaking down cell biofilms and damaging membrane integrity [34].

Results in Figure 6 showed the antibiofilm effect of purified bacteriocin, with the clear inhibition of *P. aeruginosa* biofilm observed when using bacteriocin compared with the control.

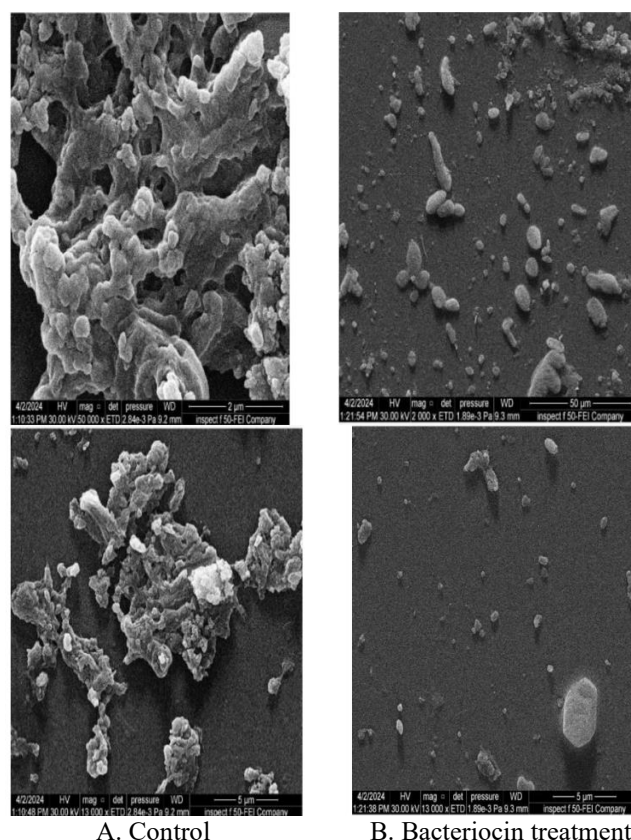


Figure 6. Detecting the effect of bacteriocin purified from *Leuconostoc mesenteroides* ssp. *cremoris* on *P. aeruginosa* biofilm by scanning electron microscope (SEM)

The results of SEM analysis showed that sonorensin not only inhibited biofilm formation but also reduced the thickness of mature biofilms [27, 35]. Also indicated using SEM detection that the cells have lost their original shapes through damage to the cell wall, which leads to the appearance of wrinkles, deformation, and decomposition of the bacterial cell. The recent study [36] shows the SEM detection. The two 16 mg/L and 32 mg/L concentrations of bacteriocin doses significantly affected the biofilms after a 24-hour treatment, losing most of the matrix. This essential biofilm component was almost eliminated, but some of the filaments attaching the cells to the substrate were still there. The absence of matrix caused the cell surface to become relatively smooth, compared to many of the cells in untreated biofilms, which have a rough surface [37]. The bacteriocin showed potential for exploitation as an alternative to chemical preservatives or as a substitute for antibiotics [38, 39].

4. CONCLUSIONS

The bacteriocin purified from *Leuconostoc mesenteroides* subsp. *cremoris* had an inhibitory effect against the growth of *P. aeruginosa* and its biofilm and pyocyanin production, and had the ability to increase cell permeability.

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REFERENCES

- [1] Salvetti, E., Campedelli, I., Larini, I., Conedera, G., Torriani, S. (2021). Exploring antibiotic resistance diversity in *Leuconostoc* spp. by a Genome-Based Approach: Focus on the *lsaA* gene. *Microorganisms*, 9(3): 491. <https://doi.org/10.3390/microorganisms9030491>
- [2] Hitendra, J., Suvarna, V.C., Niveditha, S.B. (2015). Antimicrobial attributes of *Leuconostoc* isolates against fruit and vegetable spoilage organisms. *International Journal of Current Microbiology and Applied Sciences*, 4(11): 160-166.
- [3] Ahsan, A., Mazhar, B., Khan, M.K., Mustafa, M., Hamad, M., Ali, N.M. (2022). Bacteriocin-mediated inhibition of some common pathogens by wild and mutant *Lactobacillus* species and in vitro amplification of bacteriocin encoding genes. *ADMET and DMPK*, 10(1): 75-87. <https://doi.org/10.5599/admet.1053>
- [4] Ghapanvari, P., Taheri, M., Jalilian, F.A., Dehbashi, S., Dezfuli, A.A.Z., Arabestani, M.R. (2022). The effect of nisin on the biofilm production, antimicrobial susceptibility and biofilm formation of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *European Journal of Medical Research*, 27: 173. <https://doi.org/10.1186/s40001-022-00804-x>
- [5] Alvarez-Sieiro, P., Montalbán-López, M., Mu, D.D., Kuipers, O.P. (2016). Bacteriocins of lactic acid bacteria: Extending the family. *Applied Microbiology and Biotechnology*, 100(7): 2939-2951. <https://doi.org/10.1007/s00253-016-7343-9>
- [6] Ahmad, V., Khan, M.S., Jamal, Q.M.S., Alzohairy, M.A., Al Karaawi, M.A., Siddiqui, M.U. (2017). Antimicrobial potential of bacteriocins: In therapy, agriculture and food preservation. *International Journal of Antimicrobial Agents*, 49(1): 1-11. <https://doi.org/10.1016/j.ijantimicag.2016.08.016>
- [7] Arakawa, K., Yoshida, S., Aikawa, H., Hano, C., Bolormaa, T., Burenjargal, S., Miyamoto, T. (2016). Production of a bacteriocin - like inhibitory substance by *Leuconostoc mesenteroides* subsp. *dextranicum* 213M0 isolated from Mongolian fermented mare milk, airag. *Animal Science Journal*, 87(3): 449-456. <https://doi.org/10.1111/asj.12445>
- [8] Todorov, S.D., de Melo Franco, B.D.G., Tagg, J.R. (2019). Bacteriocins of Gram-positive bacteria having activity spectra extending beyond closely-related species. *Beneficial Microbes*, 10(3): 315-328.
- [9] Yi, E.J., Kim, A.J. (2023). Antimicrobial and antibiofilm effect of bacteriocin-producing *Pediococcus inopinatus* K35 isolated from kimchi against multidrug-resistant *Pseudomonas aeruginosa*. *Antibiotics*, 12(4): 676. <https://doi.org/10.3390/antibiotics12040676>
- [10] Yin, R., Cheng, J.L., Wang, J.Y., Li, P.X., Lin, J.S. (2022). Treatment of *Pseudomonas aeruginosa* infectious biofilms: Challenges and strategies. *Frontiers in Microbiology*, 13: 955286. <https://doi.org/10.3389/fmicb.2022.955286>
- [11] Phan, S., Feng, C.H., Huang, R., Lee, Z.X., Moua, Y., Phung, O.J., Lenhard, J.R. (2023). Relative abundance and detection of *Pseudomonas aeruginosa* from chronic wound infections globally. *Microorganisms*, 11(5): 1210. <https://doi.org/10.3390/microorganisms11051210>
- [12] Vidailiac, C., Chotirmall, S.H. (2021). *Pseudomonas aeruginosa* in bronchiectasis: Infection, inflammation, and therapies. *Expert Review of Respiratory Medicine*, 15(5): 649-662. <https://doi.org/10.1080/17476348.2021.1906225>
- [13] Azam, M.W., Khan, A.U. (2019). Updates on the pathogenicity status of *Pseudomonas aeruginosa*. *Drug Discovery Today*, 24(1):350-359. <https://doi.org/10.1016/j.drudis.2018.07.003>
- [14] Feretzakis, G., Loupelis, E., Sakagianni, A., Skarmoutsou, N., et al. (2019). A 2-year single-centre audit on antibiotic resistance of *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae* strains from an intensive care unit and other wards in a general public hospital in Greece. *Antibiotics*, 8(2): 62. <https://doi.org/10.3390/antibiotics8020062>
- [15] Kumar, A., Alam, A., Rani, M., Ehtesham, N.Z., Hasnain, S.E. (2017). Biofilms: Survival and defense strategy for pathogens. *International Journal of Medical Microbiology*, 307(8): 481-489. <https://doi.org/10.1016/j.ijmm.2017.09.016>
- [16] Halami, P.M., Badarinath, V., Manjulata Devi, S., Vijayendra, S.V.N. (2011). Partial characterization of heat-stable, antilisterial and cell lytic bacteriocin of *Pediococcus pentosaceus* CFR SIII isolated from a vegetable source. *Annals of Microbiology*, 61: 323-330. <https://doi.org/10.1007/s13213-010-0145-x>
- [17] Danial, E.N., Al-Zahrani, S.H.M., Al-Mahmoudi, Z.A.H.M. (2020). Partial purification, characterization and antibacterial activity of bacteriocin from *leuconostoc mesenteroides*. *International Journal of Lifescience and*

- Pharma Research, 10(3): L6-13. <https://doi.org/10.22376/ijpbs/lpr.2020.10.3.L6-13>
- [18] Golneshin, A., Gor, M.C., Williamson, N., Vezina, B., Van, T.T.H., May, B.K., Smith, A.T. (2020). Discovery and characterisation of circular bacteriocin plantacyclin B21AG from *Lactiplantibacillus plantarum* B21. *Heliyon*, 6(8): e04715. <https://doi.org/10.1016/j.heliyon.2020.e04715>
- [19] Masuda, Y., Ono, H., Kitagawa, H., Ito, H., Mu, F.Q., Sawa, N., Zendo, T., Sonomoto, K. (2011). Identification and characterization of leucocyclicin Q, a novel cyclic bacteriocin produced by *Leuconostoc mesenteroides* TK41401. *Applied and Environmental Microbiology*, 77(22): 8164-8170. <https://doi.org/10.1128/AEM.06348-11>
- [20] Indira, M., Venkateswarulu, T.C., Prabhakar, K.V., Peele, K.A., Krupanidhi, S. (2018). Isolation and characterization of bacteriocin producing *Enterococcus casseliflavus* and its antagonistic effect on *Pseudomonas aeruginosa*. *Karbala International Journal of Modern Science*, 4(4): 361-368. <https://doi.org/10.1016/j.kijoms.2018.09.002>
- [21] Haghighatafshar, H., Talebi, R., Tukmechi, A. (2021). The effect of bacteriocin isolated from *Lactobacillus rhamnosus* on *Pseudomonas aeruginosa* lipopolysaccharides. *Avicenna Journal of Clinical Microbiology and Infection*, 8(2): 45-50. <https://doi.org/10.34172/ajcmi.2021.09>
- [22] Shams Eldeen, M.A., Elnahal, A., Ads, A., Elsawaf, M., Samy, S.M. (2021). Effect of *Lactobacillus plantarum* on virulence factors of *Pseudomonas aeruginosa* isolated from wound infection. *Microbes and Infectious Diseases*, 2(4): 790-796.
- [23] Erkaya, E., Genc, B., Akbulut, S., Adiguzel, G., Omeroglu, M.A., Ozkan, H., Adiguzel, A. (2020). Bacteriocin producing bacteria isolated from Turkish traditional sausage samples. *Journal of Pure and Applied Microbiology*, 14(2): 1567-1576. <https://doi.org/10.22207/JPAM.14.2.55>
- [24] Sharma, V., Harjai, K., Shukla, G. (2018). Effect of bacteriocin and exopolysaccharides isolated from probiotic on *P. aeruginosa* PAO1 biofilm. *Folia Microbiologica*, 63: 181-190. <https://doi.org/10.1007/s12223-017-0545-4>
- [25] Cavera, V.L., Arthur, T.D., Kashtanov, D., Chikindas, M.L. (2015). Bacteriocins and their position in the next wave of conventional antibiotics. *International Journal of Antimicrobial Agents*, 46(5): 494-501. <https://doi.org/10.1016/j.ijantimicag.2015.07.011>
- [26] Timothy, B., Iliyasu, A.H., Anvikar, A.R. (2021). Bacteriocins of lactic acid bacteria and their industrial application. *Current Topics in Lactic Acid Bacteria and Probiotics*, 7(1): 1-13. <https://doi.org/10.35732/ctlabp.2021.7.1.1>
- [27] Zhao, X.C., Zhen, Z., Wang, X.Y., Guo, N. (2017). Synergy of a combination of nisin and citric acid against *Staphylococcus aureus* and *Listeria monocytogenes*. *Food Additives & Contaminants: Part A*, 34(12): 2058-2068. <https://doi.org/10.1080/19440049.2017.1366076>
- [28] Cruz, V.L., Ramos, J., Martinez-Salazar, J., Montalban-Lopez, M., Maqueda, M. (2021). The role of key amino acids in the antimicrobial mechanism of a bacteriocin model revealed by molecular simulations. *Journal of Chemical Information and Modeling*, 61(12): 6066-6078. <https://doi.org/10.1021/acs.jcim.1c00838>
- [29] Wang, Y., Fu, X.J., Wang, Y., Wang, J., Kong, L.C., Guo, H.Y. (2024). Antibacterial activity and cytotoxicity of the novel bacteriocin Pkmh. *International Journal of Molecular Sciences*, 25(17): 9153. <https://doi.org/10.3390/ijms25179153>
- [30] Lee, D.H., Kim, B.S., Kang, S.S. (2020). Bacteriocin of *Pediococcus acidilactici* HW01 inhibits biofilm formation and virulence factor production by *Pseudomonas aeruginosa*. *Probiotics and Antimicrobial Proteins*, 12: 73-81. <https://doi.org/10.1007/s12602-019-09623-9>
- [31] Field, D., Seisling, N., Cotter, P.D., Ross, R.P., Hill, C. (2016). Synergistic nisin-polymyxin combinations for the control of *Pseudomonas* biofilm formation. *Frontiers in Microbiology*, 7: 1713. <https://doi.org/10.3389/fmicb.2016.01713>
- [32] Sharma, S., Mohler, J., Mahajan, S.D., Schwartz, S.A., Bruggemann, L., Aalinkel, R. (2023). Microbial biofilm: A review on formation, infection, antibiotic resistance, control measures, and innovative treatment. *Microorganisms*, 11(6): 1614. <https://doi.org/10.3390/microorganisms11061614>
- [33] Shokri, D., Khorasgani, M.R., Mohkam, M., Fatemi, S.M., Ghasemi, Y., Taheri-Kafrani, A. (2018). The inhibition effect of lactobacilli against growth and biofilm formation of *Pseudomonas aeruginosa*. *Probiotics and Antimicrobial Proteins*, 10: 34-42. <https://doi.org/10.1007/s12602-017-9267-9>
- [34] Yu, W., Guo, J.Q., Liu, Y.Y., Xue, X.G., Wang, X.R., Wei, L.L., Ma, J.G. (2023). Potential impact of combined inhibition by bacteriocins and chemical substances of foodborne pathogenic and spoilage bacteria: A review. *Foods*, 12(16): 3128. <https://doi.org/10.3390/foods12163128>
- [35] Chopra, L., Singh, G., Kumar Jena, K., Sahoo, D.K. (2015). Sonorensin: A new bacteriocin with potential of an anti-biofilm agent and a food biopreservative. *Scientific Reports*, 5(1): 13412. <https://doi.org/10.1038/srep13412>
- [36] Velázquez-Suárez, C., Cebrián, R., Gasca-Capote, C., Sorlózano-Puerto, A., Gutiérrez-Fernández, J., Martínez-Bueno, M., Maqueda, M., Valdivia, E. (2021). Antimicrobial activity of the circular bacteriocin AS-48 against clinical multidrug-resistant *Staphylococcus aureus*. *Antibiotics*, 10(8): 925. <https://doi.org/10.3390/antibiotics10080925>
- [37] Tawakol, M., Nabil, N., Reda, R. (2018). Molecular studies on some virulence factors of *Pseudomonas aeruginosa* isolated from chickens as a biofilm forming bacteria. *Assiut Veterinary Medical Journal*, 64(159): 43-51.
- [38] Cox, G., Stogios, P.J., Savchenko, A., Wright, G.D. (2015). Structural and molecular basis for resistance to aminoglycoside antibiotics by the adenylyltransferase ANT (2'-)-Ia. *MBio*, 6(1): e02180-14. <https://doi.org/10.1128/mbio.02180-14>
- [39] DeBritto, S., Gajbar, T.D., Satapute, P., Sundaram, L., Lakshmikantha, R.Y., Jogaiah, S., Ito, S.I. (2020). Isolation and characterization of nutrient dependent pyocyanin from *Pseudomonas aeruginosa* and its dye and agrochemical properties. *Scientific Reports*, 10(1): 1542. <https://doi.org/10.1038/s41598-020-58335-6>