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# Effect of *Lactobacillus* spp. bacteria on expression of pyocyanin genes in *Pseudomonas aeruginosa* isolated from clinical sources

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## 1.1 Introduction

*Pseudomonas aeruginosa* is Gram-negative bacilli inhabit diverse environmental conditions and therefore it can be isolated from various living and unliving sources. *P. aeruginosa* is an opportunistic pathogenic bacterium involved in many infections worldwide. Among opportunistic pathogenic bacteria and due to produce distinct virulence factors, *P. aeruginosa* causes respiratory infections, urinary tract infections, hospital acquired pneumonia, wound and soft tissue infections. Also, it causes bacteremia in immunocompromised patients, including patients with thermal injuries, keratitis, otitis externa, and folliculitis (Morita *et al*., 2014; Moradali *et al*., 2017; Pachori *et al*., 2019).

*P. aeruginosa* strains have improved resistance against broad spectrum of antibiotics such as fluoroquinolones that widely used for curing the infections in hospitals. It is known that these bacteria are capable of acquiring resistance during antibiotic therapy (Pang *et al*., 2018). This aggressive behaviour is due to many reasons included several mechanisms for virulence factors, adaptation, survival in low nutrient environments and resistance of different antibiotics classes (Moradali, 2017).

*P. aeruginosa* formed biofilm on biotic and abiotic surfaces. Most of biofilms matrix embedding bacterial cells may report over 90% of dry weight of the whole biofilm mass. In fact, the biofilm matrix provides suitable environments for survive and maintain the cells, while shielding them from unfavorable conditions such antibiotics (Chan *et al*., 2021).

Bacterial pigments are another factors that confer the microbe severity during the infections. A large number of bacteria produce pigments which are assigned different functions, such as photosynthesis, protection against ultraviolet radiation, iron uptake, ecological interactions with other organisms and participation in the signaling that modulates gene expressions dependent on cell density (Gonçalves *et al*., 2021). *P. aeruginosa*, like other bacteria, produce

colored substances as soluble extracellular nitrogenous heterocyclic compounds called phenazines. Pyocyanin, a redox-active blue-green compound, is one of the most important among phenazines pigments. Most of *P. aeruginosa* strains produce one or more of these pigments (Cohen *et al*., 2017; Riedel *et al*., 2019). Synthesis and secretion of pyocyanin contributes significantly to bacterial infection and makes *P. aeruginosa*, more aggressive (Zhou *et al*., 2022). The cytotoxicity of pyocyanin is attributed to its ability to generate reactive oxygen species (ROS) in particular $H_2O_2$ that causes oxidative stress in biological systems (Rashid *et al*., 2022).

Secretion of virulence factors by this bacterium is controlled by a mechanisms called Quorum sensing (QS) by which bacteria can communicate with the same species or other bacteria/microbes in order to achieve a specific process associated with the virulence. For example, induce biofilm formation that protects them from dryness, pressure, and immune cells. QS network produced by *P. aeruginosa* is also responsible for pyocyanin production and other virulence factors, which makes the bacteria highly drug-resistant (Pachori *et al*., 2019; Tang *et al*., 2021; Lim *et al*., 2022). Biosynthesis of pyocyanin is genetically controlled by several genes included *phzM*, *phzS*, and *rhlR*, and two identically organized operons that encode the enzymes that make pyocyanin (Askitosari *et al*., 2019).

To overcome the pathogenicity of *P. aeruginosa*, there are some strategies can be applied act as anti-virulence agents. These agents could be work synergistically with antibiotics to treat the infection by this bacterium. For example, nanoparticles, medicinal plant extract and the secondary metabolite derived from microorganisms. In this regard, the metabolites produced from probiotic bacteria, especially *Lactobacillus* spp. can be used to suppress some virulence factors formation like pyocyanin as it will be discussed later. Probiotics are live microorganisms that give the host a health advantage when given in sufficient doses. In terms of potential therapies for human health,

## Summary

*Pseudomonas aeruginosa* is bacilli an opportunistic pathogenic bacterium inhabits diverse environmental conditions. It causes severe infections due to produce distinct virulence factors such as blue-green pyocyanin. This pigment confers the microbe severity during the infections. This study aimed to evaluate metabolites from *Lactobacillus* on pyocyanin production. Two hundred twenty (220) samples were obtained from different clinical human sources including sputum, wounds, burns, urine and ears from November, 2021 to March, 2022. The samples were collected in the laboratories of Baqubah Teaching Hospital, Diyala Province, Iraq. The samples were cultured using differentiate and selective culture media. Thirty-two isolates of *P. aeruginosa* were identified and confirmed by microscopic technique, biochemical test, API20E assay and VITEK2 system. *P. aeruginosa* isolates were subjected to Kirby-Bauer test to examine their antibiotic susceptibility towed 12 different standard antibiotics including Piperacillin/Tazobactam, Ticarcillin/clavulanic acid, Ceftazidime, Ciprofloxacin, Gentamicin, Norfloxacin, Ceftriaxone, Amikacin, Netilmicin, Aztreonam, Imipenem and Meropenem belong to six different classes. The antibiotic resistance of the isolates was 18.75%, 100%, 100%, 15.62%, 28.12%, 100%, 15.62%, 12.5%, 25%, 28.12%, 18.75%, 68.75%, respectively.

The ability of the isolates to form biofilm was confirmed by microtitre plate method. The results showed that 43.7% of the tested isolates were strong biofilm producers, while 46.8% were average, and 9.3% of them did not produce biofilm. The colorimetric method that used to determine the Quorum Sensing (QS) signal molecules N-Acyl homoserine lactones (AHL) revealed that all the isolates were producing AHL with absorbance (630 nm) ranged between 2.27 to 4.90. All *P. aeruginosa* isolates were produced pyocyanin dye when tested colorimetric and the highest dye-producers was obtained from burns. The rate of pyocyanin production ranged between 0.47 to 0.82 µg/ml.

lactobacilli have received the greatest attention among the various microbial species. Traditional starter cultures for the production of fermented foods use (lactobacilli), which are naturally present in the human gastrointestinal and urogenital tract microbiota (Pradhan *et al*., 2020). Many mechanisms could be behind the activity of probiotics as anti-infective uncovered throughout the years. Many members of probiotics can also create a wide range of bioactive compounds that are comparable to the standard antibiotic. Bacteriocins, organic acid, hydrogen peroxide, diacetyl, acetoin is an example of these materials (Santacroce *et al*., 2019).

## 1.2 The aim of study

The main goal of this project was to find way to reduce or inhibit the virulence factor, pyocyanin pigment, in *P. aeruginosa* by the use of probiotic bacteria as an alternative to conventional agents. Here, we evaluate the efficacy of probiotic extracts against *P. aeruginosa* in vitro. In order to achieve this, five objectives were studied:

1) Isolation and identification a clinical isolates of *P. aeruginosa* from different clinical sources.
2) Isolation and identification of probiotic bacteria from different sources.
3) Investigate the antibiotic resistance profile of *P. aeruginosa* isolates.
4) Determination the inhibitory effect of probiotic bacteria extracts on pyocyanin production in *P. aeruginosa* isolates
5) Detection of gene expression of pyocyanin encoded gene in *P. aeruginosa* treated with probiotic extract.
6) Study the expression of some genes that affect pyocyanin production after treatment with *Lactobacillus* spp.