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Genetic detection and phenotypic detection of the mutant S_3RB gene using mutagens by radioactive sources, lasers and magnetic water encoded into mutant pyocin produced from mutant *Pseudomonas aeruginosa*

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Abstract

Objective: The aim of this research is a genetic and phenotypic study of the mutant *S_3RB* gene encoded to pyocin (bacteriocin) from mutant *Pseudomonas aeruginosa* after exposure to physical mutagens.

Study design: Cross-sectional in descriptive and case–control in analytical study design.

Backgrounds: *P. aeruginosa* is gram negative bacteria, a multi-drug resistance (MDR), profiteer pathogen occasion several diseases acute, chronic contagion in immunodeficiency patients to chronic obstructive pulmonary disease (COPD), cystic fibrosis, cancer, sepsis, burns, traumas and COVID-19. Utmost strains production bacteriocin able of prohibit another strains known pyocin that are classified to kinds inclusive R- type, F-type, S-type and others.

Results: Results of phenotypic detection of mutant pyocin for (16) isolates of mutant *P. aeruginosa* after mutagenesis

Methodology: Study patients, bacterial strain, media and growth conditions of *Pseudomonas aeruginosa. Exposure to* physical mutagenesis by physical mutagens. Phenotypic of pyocin (bacteriocin) before and after mutagenesis. Genotypic detection of pyocin (bacteriocin) before and after mutagenesis by PCR device.

through formation large inhibition zone against *Staphylococcus aureus* around the hole (contain *P. aeruginosa*). In molecular genetics results of genotypic detection by PCR of pyocin produced from *P. aeruginosa* include positive results with two band size of product of *S3RB* (451 with 600) bp. in No. 1, 7, 8, 10, 11, 17, 19, 20 and 21. Also, positive results of one band for the same gene (451) bp. in No. 2, 3, 5, 9, 15 and 16, but negative results in No.4, 6, 12, 13, 14, 18, 22 and 23.

Conclusion: Cs^{137}, Co^{60}, Na^{23} and Sr^{90} radioactive sources without aluminum led to a mutation in the *S3RB* gene encoding pyocin in *P. aeruginosa*. Innuendo to radiation released of a Cs^{137} irradiant exporter in the presence of aluminum led to a mutation. The best physical mutagen for the *S3RB* gene is radiation emitted from radioactive sources.

Keywords: Bacteriocin, PCR, Bacteria, Molecular genetics, Mutagenesis, Physical mutagens and Mutation

Introduction

Pseudomonas aeruginosa is a multi-drug resistance (MDR), it is gram-negative bacilli, utmost strains produce bacteriocin [1]. opportunistic pathogens due to severe or recurrent contagion in immunodeficiency patients to cancer, traumas and sepsis [2, 3]. Almost 700,000 patients died of the antibiotic reluctance bacteria every year [4].

Pyocin production is a settled feature that is not wasted on refined sub-culture or lengthened store from *P. aeruginosa*. Utmost strains output pyocin that prohibit the outgrowth of another strains of the same species that headmost qualified via Jacob (1954) and deliberate in detail via Hamon (1956) that belong to the class of bacteriocin. Specific strains of *P. aeruginosa* produce restirant to pyocin such as proteolytic enzymes [5].

François Jacob was the headmost depict a bacteriocin instituted of *P. aeruginosa* through treated to a mutagen via ultraviolet radiation for wavelength 253.7 nm. Pyocinogeny, capability the bacteria to output pyocin is prevalent in *P. aeruginosa*, however, naturalistic output plane of pyocin is depressed. Remediation of the cultivation via mutagenic factor due to raises the output average of pyocin [6] whilst Kageyama induce produce of pyocin through append to 1–2 μg/ml mitomycin C in boost cultures [7, 8, 9].

Bacteriocin generated by *P. aeruginosa* able of restrain another strains known pyocin that are categorized to R-type pyocin, F-type-pyocin, S-type- pyocin in order that Snippets in nitrogen base. R-type pyocin are capable to murder another gram-negative bacteria [10].

Methodology

Study design

Case-Control depending in this seek for analytical study design and Cross-Sectional for descriptive study design.

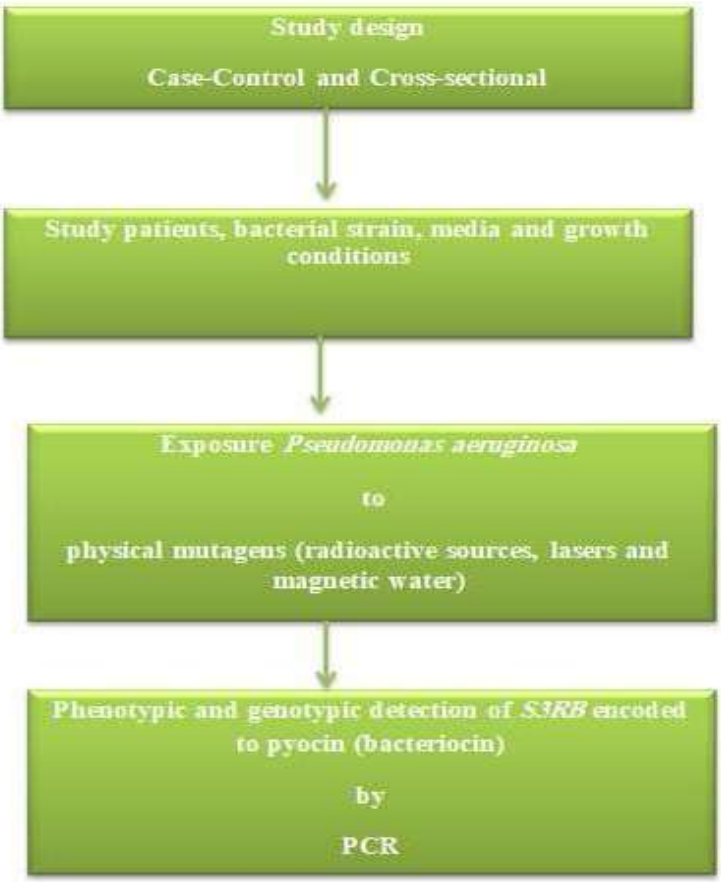


Figure (1): Plan of study design of this research.

Patients, bacterial strains, media and growth conditions

Materials and methods

A total of collecting of sample (200) *P. aeruginosa* from patients who were admitted in Baghdad infirmary in 2023/ 2024 that identified by conventional and molecular detection depend on [11].

Exposure *P. aeruginosa* to radioactive sources, lasers and magnetic water

The procedure done according to [12] with modifications begin *P. aeruginosa* bacteria were exposed to different physical mutagens and different doses of cesium radioactive sources in the subsistence and obscurity of aluminum. The bacteria were furthermore, exposed to different types of radiation emitted from lasers at different times and to magnetic water of different sizes, and to a chemical substance. The number of living cells and the percentage of killing were calculated before and after exposure as in the equation below:

$$\textbf{Kill rate} = \frac{\text{untreated- treated}}{\text{Untreated}} * 100$$

Phenotypic detection of pyocin produced from mutant *P. aeruginosa*

Pyocin production was detected by growing antibiotic-resistant Pseudomonas bacteria and cultured on Trypticase soy broth medium and subsequently brood for 24 hr. at 37°C, thereafter the tubes were centrifuged at 4000 rpm for ten minutes. Mueller-Hinton medium was prepared and two holes were made in the medium and Staphylococcus bacteria were cultured on the medium and 100 microliters of the filtrate were placed and in the second hole 100 microliters of the precipitate were placed and subsequently brood for 24 hr. near 37°C, positive results regarding to pyocin production were indicated by the presence of an inhibition radius around the holes and negative results were indicated by the absence of inhibition zones around the holes [13].

Molecular detection of *S3RB*

DNA extraction

DNA extraction accomplished according to company instructions.

Genotypic detection of *S3RB* encoded to pyocin from mutant *P. aeruginosa*

The genetic detection of *S3RB* which responsible for pyocin production by PCR (Polymerase Chain Reaction) accomplished by the oligonucleotide PCR primer used in PCR amplification were specific for detection S3RB-F (CGTATCACGAGACAGGCA) and S3RB-R (TGCCGCTTCTT CCGCTTT), the size of gene is 451 bp. compared with DNA ladder 1000 bp.[14].

Table (1): Sequences primer with size of product.

No.	Sequences of primer	Encoded for	Size of product	References
1	5′-CGTATCACGAGACAGGCA-3′	S3RB-F	451	14
2	5′- TGCCGCTTCTTCCGCTTT-3′	S3RB-R		

PCR amplification of *S3RB*

PCR mixture was prepared depend on table (2) and amplify the *S3RB* gene according to table (3).

Table (2): PCR mixture for amplification of *S3RB*.

No.	Elements	Quantity 25 (µl)
1	Green master mix	12.5
2	Forward Primer (10 Pmol)	1.5
3	Reverse Primer (10 Pmol)	1.5
4	DNA template	5
5	Nuclease free water	4.5
Total volume		**25**

Table (3): PCR program for amplification S3RB encoded for pyocin.

No.	Steps	Temperatures
1	Denaturation	95 ºC
2	Annealing	58 ºC
3	Elongation	72 ºC
4	Final elongation	72 ºC
Cycle= 35		

PCR output were detached through agarose gel electrophoresis at 1% agarose gel while rapprochement to 100 bps. DNA ladder for a measure marker beneath UV light apparatus [15, 16].

Results and discussions

Phenotypic detection of pyocin produced from mutant *P. aeruginosa*

Phenotypic detection for production pyocin of (200) isolates of *P. aeruginosa*. The results have been (50) isolates produce pyocin by inhibition zone around the hole before the mutagenesis by physical mutagens. Also, (16) isolates of *P. aeruginosa* produce pyocin after mutagenesis through formation large inhibition zone against *Staphylococcus aureus* around the hole (contain *P. aeruginosa*) as shown in figure (2).

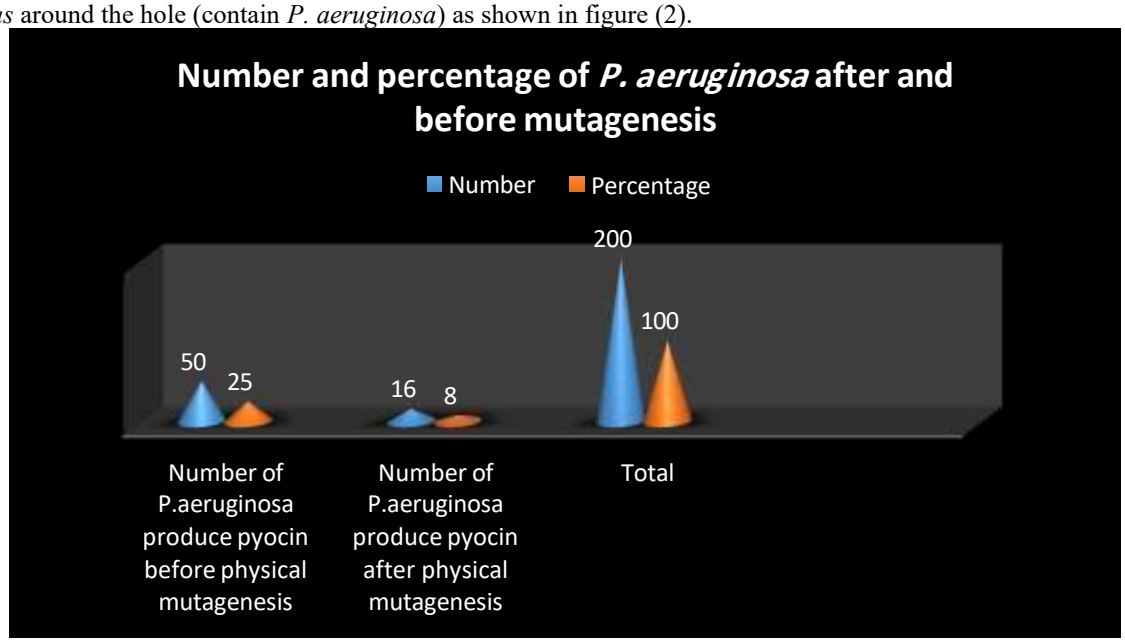


Figure (2): Number and percentage of *P. aeruginosa* after and before mutagenesis.

Genotypic detection of *S3RB* encoded to pyocin from mutant *P. aeruginosa*

Results in table (4) and figure (3) show positive with two bands in different size 451 and 600 bp. for *S3RB* of *P. aeruginosa* of isolates No. 1, 7, 8, 10, 11, 17, 19, 20 and 21. This results indicates present mutation in *P. aeruginosa*. Also, positive with one band of size 451 bp. for *P. aeruginosa* of isolates No. 2, 3, 5, 9, 15 and 16. Negative results for *P. aeruginosa* of isolates No.4, 6, 12, 13, 14, 18, 22 and 23.

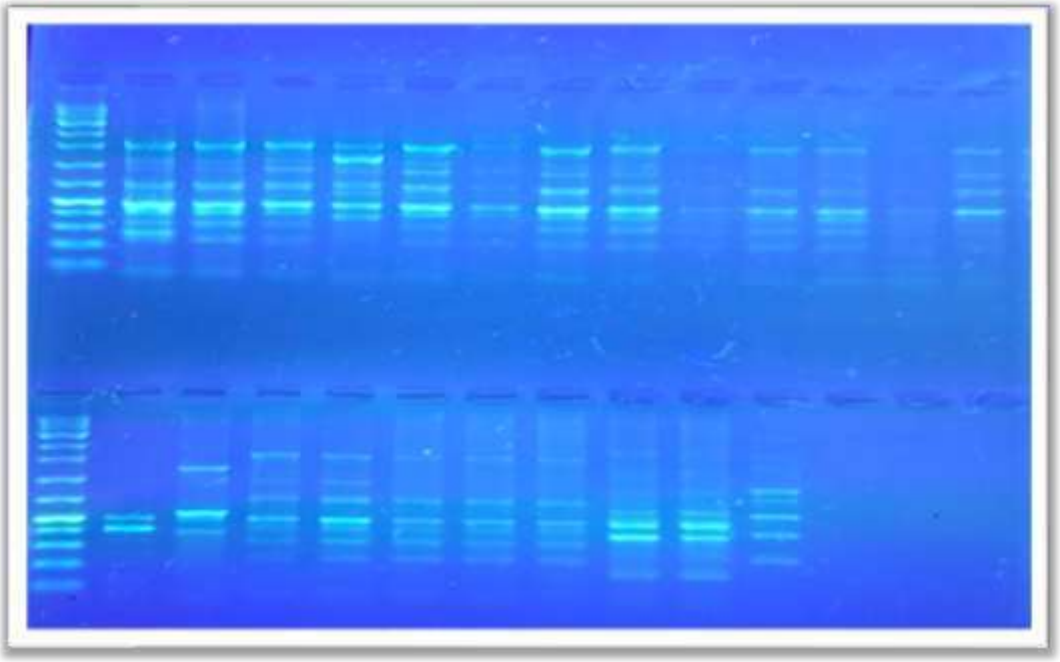


Figure (3): PCR product of mutant *S3RB* gene encoded for mutant pyocin produced from mutant *P. aeruginosa* with size 451 bp. compared with DNA ladder as marker 1000 bp.

The results in table (4) showed that exposition to ray released of radiant exporter Cs^{137}, Co^{60}, Na^{23} and Sr^{90} without aluminum led to a mutation in the *S3RB* gene encoding pyocin and the appearance of 2 bands of different sizes, 451 and 600 base pairs. Likewise, exposure to cesium in the presence of aluminum led to the appearance of 2 bands of different sizes, 451 and 600 base pairs.

Table (4): Results for PCR product of *S3RB* gene of *P. aeruginosa* after exposure to physical mutagenesis.

No.	Results Positive/ Negative	Size of product	Type of mutagens
1	Positive	451+600	Na^{23} with activity 10 µci for 3 hr. without alminium
2	Positive	451	Na^{23} with activity 10 µci for 2 hr. without alminium
3	Positive	451	Na^{23} with activity 10 µci for 1 hr. without alminium
4	Negative	……	8 ml from Magnetic water exposed for 1 hr.
5	Positive	451	10 ml from Magnetic water exposed for 1 hr.
6	Negative	……	Co^{60} with activity 1 µci for 3 hr. without alminium
7	Positive	451+600	Co^{60} with activity 10 µci for 3 hr. without alminium
8	Positive	451+600	Cs^{137} with activity 10 µci for 1 hr. without alminium
9	Positive	451	Cs^{137} with activity 10 µci for 1 hr. with alminium
10	Positive	451+600	Cs^{137} with activity 10 µci for 3 hr. with alminium
11	Positive	451+600	Cs^{137} with activity 10 µci for 3 hr. without alminium
12	Negative	……	Laser in 10 min
13	Negative	……	Laser in 20 min
14	Negative	……	Chemical substances in 3 g.
15	Positive	451	8 ml from Magnetic water exposed for 1 hr.
16	Positive	451	Cs^{137} with activity 1 µci for 2 hr. with alminium
17	Positive	451+600	Cs^{137} with activity 10 µci for 2 hr. without alminium
18	Negative	……	Co^{60} with activity 10 µci for 2 hr. with alminium
19	Positive	451+600	Co^{60} with activity 1 µci for 1 hr. without alminium
20	Positive	451+600	Sr^{90} with activity 10 µci for 1 hr.
21	Positive	451+600	Sr^{90} with activity 10 µci for 2 hr.
22	Negative	……	Na^{23} with activity 10 µci for 1 hr. with alminium

23	Negative	**……**	Co^{60} with activity 10 µci for 3 hr. with alminium

From the previous study [17] the results of the phenotypic detection of pyocin produced by Pseudomonas bacteria showed positive results for 85 isolates producing pyocin and 29 isolates unable to produce pyocin.

P. aeruginosa output bacteriocin such as pyocin, especially S-type pyocin that are committed for biological enforcement. Antibacterial subaltern metabolites are assorted to two major class via inclusive minimal molecular mass like organic acids while elevated molecular mass such as bacteriocin. Bacteriocin perform a decisive function in vindicatory and competitive technicality [18].

S-pyocin are massive polypeptides with doubled scopes enclose a relative impregnability protein which disrupt the stimulant field of the efficient pyocin which are dissolvable and susceptible to heat and proteases, a feature which recognize of R- and F-pyocin. R- and F-type pyocin, furthermore called as tailocins, established as disturbed prophages to hereditary engagement to P2 and lambda phages. R-type pyocin hold astringent construction but forfeit pliability whilst F-type pyocin are pliability however forfeit contractility. Newly, M and G-pyocin are bacteriocin created via *P. aeruginosa* through the suitable particularity of pyocin are the capability to inhibit of nourishment deterioration, prohibit pathogenic microbes with display antiphlogestic and oxidation inhibitor influence [19, 20].

This study by (21) seem importance of mutagenesis in production of *P. aeruginosa* Above 60 percentage of the universal outputs of manufacturing enzymes are proteolytic enzymes almost 35 percentage are alkaline proteases. The actual microbial resources are incapable to extent industrial request of alkaline protease that lead experts to consideration novel resources with induction enzyme activity. Thence stratified UV radiation to improve a *P. aeruginosa* mutant as a novel resource of protease overoutput, treaded via cultural and nourishment optimization. Pyocins are antimicrobial bacteriocins which goal another strains of the like species, though several pyocin kinds set to target another species to *P. aeruginosa* (22). Also, pyocin output arrive with estimate due to cells which product pyocin through the SOS restraint lyse and endear. Pyocin pack to minimal grade in criterion cultures next nightly cultivation and the extension of genotoxic factors like mitomycin C (MMC) through the exponential phase robustly prompts pyocin output (23). As a result in this study (24) it was assured secluded of kimchi safely cut-price MDR *P. aeruginosa* expansion and bacterial film formulation. Thus, kimchi protrude as a possible resource of bacteria capable to assist remedy diseases confederated to antibiotic-reluctant contagion.

Conclusions

1- Cs^{137}, Co^{60}, Na^{23} and Sr^{90} radioactive sources without aluminum led to a mutation in the *S3RB* gene encoding pyocin in *P. aeruginosa*.
2- Exposure to radiation emitted from a Cs^{137}radioactive source in the presence of aluminum led to a mutation.
3- The best physical mutagen for the *S3RB* gene is radiation emitted from radioactive sources.
4- Mutant bacteriocin can use as antibiotic replacement therapy.
5- Mutant bacteriocin serve as treatment of drug-resistant bacterial infections.
6- Biotechnological applications of mutant bacteriocin as novel mutant drug against *Staphylococcus aureus*, *Enterococcus spp.*, *Klebsiella pneumonia*, *Acinetobacter baumanii*, *E. coli* and bacterial tumors (cancer).
7- Cs^{137}, Co^{60}, Na^{23} and Sr^{90} radioactive sources without aluminum led to a mutation in the *S3RB* gene encoding pyocin in *P. aeruginosa*.
8- Exposure to radiation emitted from a Cs^{137} radioactive source in the presence of aluminum led to a mutation.
9- The best physical mutagen for the *S3RB* gene is radiation emitted from radioactive sources.

Data Accessibility statement

The datasets analyzed and/or construed during the current treatise upon corresponding author to sensible request.

Consent to publish

All author approved the final manuscript for publish.

Founding and sponsorship

No founding was received for conducting this research.

Competing interest

The authors declare no competing interests.

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