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Increase production of Prodigiosin pigment from mutant *Serratia marscences* against bacterial malignant tumors (Cancers) by physical mutagenesis

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Abstract

The goal of study in order that increase expression of prodigiosin pigment from mutant *Serratia marscences* with study the effect antimicrobial activity of prodigosin pigment against bacterial isolated from malignant tumors patients. The isolates collected 125 isolates from Baghdad hospitals linked with Leukemia, Belly cancer, Cochlea cancer, Spleen cancer, Renal cancer and Liver cancer.

Outcome of isolation were 20 isolates (17%) *Staphylococcus aureus*; 18 (14%) *Escherichia coli* ; 9(7%) *Acinetobacter baumanii*; 9 (7%) *Salmonella spp*.; 9 (7%) *Streptococcus sp* .; 5 (4%) of *Klebsiella pneumonia;* 5 (4%) of *Pseudomonas aeruginosa,*; 5(4%) *Pantoea spp.;* 5(4%) *Aeromonas spp*.; 5(4%) *Morganella morganii* ; 5 (4%) *Staphylococcus epidermidis* and 5(4%) *Micrococcus sp*. , also collecting *Serratia marscences* 25(20%) from total 125 isolates from different infections of patients.

The physical mutagenesis achieved by Nd:YAG lasers in 500 pulse, the results of mutagenesis exhibit high expression of prodigosin after physical mutagenesis to isolates of *S.marscences* which was 15 isolates (60%) and compared prodigiosin production before the mutagenesis which was 25 isolates (total isolates of *S.marscences*);the bacteria were also exposed to Alpha, Gamma and Beta rays to

different radiosources (isotopes) including Am^{241}(1Mci) emitte Gamma and Alpha ray with dose $0.31993*10^{-4}$KGy for Gamma ray and 1.4157 KGy at 3 hr. for Alpha particles.; Sr^{90} (9Mci) emitte Beta ray in dose $1.973*10^{-8}$ KGy at 3 hr.; Cs^{137} (1Mci) emitte Beta and Gamma ray in dose $1.3158*10^{-8}$ KGy at 2 hr. for Beta ray and $1.973*10^{-10}$ KGy at 3 hr. for Gamma ray; Na^{22}(1Mci) emitte Beta and Gamma ray in dose $1.533*10^{-8}$ KGy at 3hr..The results of exposure showed high improvement production of prodigosin in Americium were 21 isolates (84%), Strontium 17(68%), Cesium 23(92%) and Sodium 12(48%) that give high expression of prodigosin pigment.

The prodigiosin dye was taken away of *S.marscences* via Chloroform in acid-base medium method and purification by TLC (Thin Layer Chromotography mechanization) by employ Silica gel sheet.

The antibacterial effect of antibiotics done to *S.marscences* before the physical mutagenesis and after physical mutagenesis into 28 antibiotics in order that determine susceptibility by resistance and sensitive, results the study of resistance of *S.marscences* before physical mutagenesis improved turn off into sensitive of different antibiotics were *E.coli* turn off sensitive to ciprofloxacin after physical mutagenesis, as well *A.baumanii* turn off sensitive to Amikacin, Piperacillin; *Morganella morgana*i turn off sensitive to Pipracillin; *K.pneumonia* turn off sensitive to Ciprofloxacin, *Pantoea spp*. turn off sensitive Tetracyclin; *S.aureus* turn off sensitive to Tetracyclin ; *S.epidermidis* turn off sensitive Methicillin and Streptococcus turn off sensitive Penicillin G.

The antibacterial effect of prodigiosin pigment from mutant *S.marscences* was done before the mutagenesis and after the mutagenesis in order that comparsion between them by utilizing well diffusion method, the results of antimicrobial activity of prodigosin against bacterial tumors exhibit possession effectiveness on all isolates(100 isolates) but differ in the effect of diameter of inhibition zone around well, almost possess high inhibition zone area another possession intermediate inhibition zone and others possession small inhibition zone; all the antimicrobial activity test grant positive results for killing bacterial malignant tumors (cancers).

Key words: Mutagen, Tumors, Radiation, Mutant, Lasers, Radiosources.

Introduction

Serratia spp. is a rod-form , Gram negative , aerobic or anaerobic in some situation (Bayona et al.,2009). It is motile, non spore forming, Opportunistic pathogen (Perez *et al.*,2011) causes diverse ailment inclusive Pneumonia, wound infection, Meningitis , Septicemia and infections from respiratory, urinary duct and endocarditis(Casolari *et al.*,2005; Matsuo *et al.*,2008).

Prodigiosin is a red pigment secondary metabolic product restricted to the plasma membrane of microorganisms, created during stationary phase(Wang et al.,2004;Fineran et al.,2005).It's not able to intermingle by diffusion in the environment, not resolve in water, but dissolve in alcohol agent and compartively of organic solvent inclusive bromoform, chloroform, acetone, benzene, Di methyl sulfoxide (DMSO) (Khanafari *et al.*,2006).

Nd:YAG lasers is (Neodymium-doped Yttrium aluminum Garnet) which supply the lasing efficiency in the crystal. Nd:YAG lasers issue light with a wavelength of 1064 nm, in the infrared while there are also switch near 946, 1120, 1320 and 1440 nm. Nd:YAG lasers turn on in together pulsate and persistent process (Ledon *et al.*,2012).

Nd:YAG lasers are utilized in ophthalmology to proper posterior capsular opacification, a situation that may happen after cataract operation and for peripheral iridotomy in patients in acute angle-closure glaucoma wherever it has replaced surgical to remove part of the iris. Frequency-doubled Nd:YAG lasers (wavelength 532 nm) are utilized for pan-retinal photocoagulation in patients with diabetic retinopathy,in particular state lasers as well utilized to remedy eye floaters (Kokave *et al.*,2017).

In Dentistry Nd:YAG dental lasers are utilized for soft tissue operation in the oral cavity, e.g. gingivectomy, periodontal sulcular

debridement, LANAP, pulpotomy, frenectomy, biopsy and coagulation of graft donor position [10]. In oncology, Nd:YAG lasers utilized to elminate skin cancers. They are also used to minimize benign thyroid nodules and to breakdown preliminary and secondary malignant liver damage (Pacella *et al.*,2009;Pompili *et al*.,2010).

Material and Methods:

Isolation and Identification of *Serratia marcescens*

Collection of bacterial isolate from malignant tumors including leukemia, stomach cancer, cochlea cancer. *S. marcescens* were isolated of wound, abscess, burn and urinary tract infections of Baghdad infirmay. The gathered blood and C.S.F specimens were schedule to brain heart infusion broth on MacConkey agar, Blood agar while chocolate agar.

Ready-made tape of API-20E process was utilized for the identification of the bacteria, these tape synthetic via Bio-Merieux corporation(Harly,1996). The API-20E strips contain 20 micro tubes have dehydrated substrates. The tests micro tubes were injected with bacterial hang. Through incubation, chemical process out put color variation that are either automatical or detect by addendum of reagent, as well identification of bacteria by VITEK2-GP , the Gram positive (GP) nameplate is utilized for the automated identification of utmost important non-spore-forming Gram-positive microorganisms (foremost cocci). The GP identification card is depend on determined biochemical procedures and latterly advanced substance.There are 64 biochemical experience standarize carbon source employment, enzymatic effectiveness and antibiotics impedance (Collins and Lawson,2000;Barros *et al.,*2001).

Prodigiosin production Method

1. *S. marcescens* were animated by take inoculation from original culture and mature into 5ml of brain heart infusion stock.

2. Brood at 37°C for 24 hr.-48hr.

3-The turbidity was amend approximately to 0.75 at 620 nm by utilizing spectrophotometer.

3. Whole tubing were incubated at 37C° for 72 hrs.

4. The degree to which arefractive medium retards transmitted rays of light (O.D.)was registered at 499 nm and at 620 nm.

5. The quantities of dye was studied by utilizing the equalization below.

Equation

O. D499 – (1.3831 x O.D620)

Prodigiosin U/Cell = ×1000 O. D 620

O.D499: Symbolize prodigiosin reduction

O.D620:Symbolize microorganisms reduction.

1.3831: constant.

1000: Eshew representative numbers minimal than one(Haddix and Werner,2000).

Extraction of prodigosin via chloroform into acid – base midst

1. The prodigiosin was taken away via schedule distill water into the cell hang contain *S. marcescens* that injected into Brain heart infusion stock at 30C° for 72hrs, in percent 1:1 (V/V).

2. The edification growth hodgepodge was vibrate, thereafter forsake at 6000 rpm for 15min.

3. The sediment blended together three ml of chloroform, the dye in the lower coat that detached of the upper layer.

4. 1 ml from (0.2) N HCl affix up to to the lower layer, centrifuged at 10000 rpm for 15 min.

5. 1 ml of (0.4 N) NaOH affix up to the sediment, teeming in glazier transparent dish and brood at 30C° untill 48 hrs.(Giri *et al*.,2004).

Purification of prodigiosin

1- The pigment prodigosin filtered by utilizing thin layer chromatography (TLC),the TLC from silica gel (20×20cm).

2- Solvent inclose ethyl acetate, Chloroform and acetone (65:30:5) asconform to a standard, then and teeming till the chromatography container, that satiate near animated phase. 3-3-The Rf esteem of chromatography exhibit in the TLC plates. The pigment taken away was calculated by utilizing the equalization:

Rf= $\frac{Distance\ of\ sample}{Distance\ of\ mobile\ phase}$

4-Prodigiosin Pigment rub off and render in 5ml from methanol , discard at 6000 rpm until 15 minutes to obtain liberate of silica gel leftover.
5-T he optical density metric at wave length 200-700 nm, the methanol utilized as blank.

6-The filtered Prodigosin pigment stockpiled in tube with cover of aluminum paper at 4C°(Nakashima *et al.,*2005).

Physical mutagenesis by Radiosources and ND:YAG laser

S.marscense cultivation on nutrient agar according to (Trampuz *et al.,*2006) with some modifications:
at 37° C for 24 hr. to hook up the stationary-stage growth, thereafter discard in 5000 rpm untill 10 minute. The supernatant was secluded and the precipitate was resuspended in normal saline and compared with the MacFarland solution ($1.5*10^8$ CFU/ml) , posteriorly taken 5 ml of solution was exposition to Beta, Gamma and Alpha radiation released by different isotope for various time (1,2,3,4) hr., subsequently injected at Muller Hinton Agar at 37° C for 24 hr , the colonies were

calculated and killing was specified with count perecentage of killing with neutralization below:

$$\text{Percentage of Killing } \% = \frac{\text{Control} - \text{treated}}{\text{Control}} * 100$$

Treated : Indicate to *S.aureus* treated with physical mutagenesis.

Control: : Indicate to *S.aureus* without treated with physical mutagenesis.

Antibacterial activity of Prodigosin from *S. marcescens* against bacterial cancer from malignant tumors tissue (Cup disc method)

Bacteria mature in broth culture for 24 hr., thereafter adjusted to 0.5 Macfarland spreading on Muller-Hinton agar platen. Four holes achieved almost 6mm in diameter overhead agar-paten by antiseptic cork borer. The added 100ML of prodigiosin reproducer into each well, as well control. The paten were brood at 37C° for 24 hrs, the diameter of area metric in mm(Samaranika, 2012; Bonev *et al.*,2008).

Antibacterial activity of antibiotic of mutant *S.marscences*

Kirby-Baur procedure was utilized to perform the antibiotic sensitivity test for (33) diverse antibiotics , accordingly subordinate:

1. Bacterial hang intended via selected (4-5) secluded settlement of the genuine culture and suspended to a test pipe hold 5ml of normal saline to make a bacterial hang of mild cloudiness that confront with the standard cloudiness sole.

2. By antiseptic cotton swab a fraction of bacterial suspension was transmitted and diffusion on Muller-Hinton agar.

3. The antimicrobial discs were position on the agar using a sterile forceps.

4. The paten were brood at 37C° for 24 hrs.

5. Repression area nearly the discs were metric via millimeter (mm) approbate to (CLSI,2013).

The antibiotic utilized in this research including Amikacin(AC), Amoxcillin+Clavulanic acid(AMC), Ampicillin(AMP), Azithromycin(AZT), Aztreonam(ATM), Carbenicillin(AR), Cefepime(CPM), Cefozidime(CDD), Cefurioxime(CXM), Cephalexin(CE), Ciprofloxacin(CIP),Doxycyclin(DO), Erythromycin(E), Garamycin(G), Gentamycin(GM), Imipenem(IMP), Methicillin(MET), Netilmicin(NIT), Nitrofurantion(F), Oxacillin (OX), Penicillin G(P), Piperacillin(PRL), Tetracyclin (TE), Tobramycin(TOB), Trimethoprime(TMP), Trimethoprime+ Sulphamethan(SXT) and Vancomycin(VA).

Results:

Isolation and identification of malignant tumors bacteria

Cancer cells is undifferentiated or abnormal cell, uncontrolled growth cell discordant continual to take shape abnormal mass known tumor. The Infections connected with cancers inclusive viruses, bacteria and schistosomes related to elevated hazard of malignancy(Kuper et al.,2000).

Table(1): Types of cancers and types of bacterial isolates from malignancy tumors

No.	Types of bacterial isolates from malignancy tumors	Types of cancer
1	*Acinetobacter baumanii*	Leukemia, Cochlea cancer
2	*Klebsiella pneumoniae*	Leukemia
3	*Escherichia coli*	Leukemia
4	*Pseudomonas aeruginosa*	Leukemia
5	*Staphylococcus aureus*	Leukemia,Spleen cancer

6	*Pantoea sp.*	Leukemia
7	*Salmonella sp.*	Leukemia
8	*Micrococcus sp.*	Leukemia
9	*Staphylococcus epidermidis*	Leukemia
10	*Streptococcus sp.*	Leukemia,Stomach cancer
11	*Morganella morgani*	Leukemia,Kidney cancer
12	*Escherichia coli*	Leukemia
13	*Aeromonas hydrophilia*	Leukemia,Liver cancer

Result of gathering of bacterial cancer showed *S.aureus* , *E.coli* , represents the utmost prevalent pathogenic secluded bacterium, then *S.epidermidis, A. baumanii* , *Salmonella sp., Klebsiella pneumonae, Streptococcus sp., Pantoea sp., Aeromonas sp., Pseudomonas aeruginosa, Morganella morgana* and *Micrococcus sp.* Consequently.

Serratia marcescens collected from patients with several infections from sputum, urine and wound infections, then cultivating on blood agar and maConkey agar paten.

The investigator (Zorgani *et al.,*2010) exhibit Gram negative bacteria prevailing organisms related with contagion to cancer patient inclusive *E. coli*, *K. pneumoniae* and *P. aeruginosa.*

Aprevious study by (Labour and Welfare,2003) showed type of bacteria diverge relying on type of cancer and gender and geographical allocation such as the prostate cancer in man and breast cancer in women, thereafter lung cancer, colon, rectal cancer untill both men and women, bladder, leukemia lymphoma cancer for men, ovary, stomach and liver cancer in Asian country.

The study by(Parkin *et al.,*2005) lung cancer comes in first go a head via stomach and liver cancer, colon , anal and mamma cancer.

The infections by viruses correlating with cancers and *Helicobacter pylori* cause stomach cancer and lymphoma mucosa (associated lymphoid tissue (MALT)), the second of bacterial cancers *Salmonella typhi* cause gallbladder cancer, the third of bacterial cancers by *Chlamydia pneumonia* and *Mycobacterium tuberclusis* that occasion malignant disease with lung carcinoma that higher risk cancer (Song *et al.,*2006).

Physical mutagenesis by Radiosources and Nd:YAG Lasers

Mutagenesis of *S.marscense* by Gamma, Alpha and Beta physical mutagen from diverse radiosources showed in table(2).

Table (2): Physical mutagenesis of bacterial malignancy tumors by different radiosources.

No.	Radioactive Sources	Symbol	Activity	Production date	Half -life	Type of radiation
1	$^{137}CS_{82}$	PC 95	1 Mci	1/3/1982	30.07 Y	I**γ** I**β**
2	$^{90}Sr_{52}$	S 5780	3 Mci	16/5/1978	28.79 Y	I**β**
3	$^{90}Sr_{52}$	BG 525	9.243 Mci	1/2/1999	28.79 Y	I**β**
4	$^{241}Am_{146}$	S 5298	9 Mci	10/1/1981	432.2 Y	I**γ** I***α***
5	Na^{22}	1*410	1 Mci	1/1/2008	2.6Y	I**γ** I**β**

Isotope	**Type of decaye**	**Dose kGy**	**Killing ration %**	**Time to exposition**
^{90} Sr	**β**	$1.973*10^{-8}$	89%	3hr.
^{137} Cs	ɣ	$1.973*10^{-10}$	80%	3hr.
	β	1 $.3158*10^{-8}$	86%	2hr.
	ɣ	$0.31993*10^{-4}$	90%	3hr.

^{241}Am	α	1.4157	93%	
Na^{22}	β	$1.533*10^{-8}$	91%	3hr.
	Control=300			

Results of physical mutagenesis via exposition to Alpha, Gamma and Beta rays to diverse radiosources (isotopes) present in table(2) inclusive Am^{241}(1Mci) emitte Gamma and Alpha ray with dose $0.31993*10^{-4}$KGy for Gamma ray and 1.4157 KGy at 3 hr. for Alpha particles.; Sr^{90} (9Mci) emitte Beta ray in dose $1.973*10^{-8}$ KGy at 3 hr.; Cs^{137} (1Mci) emitte Beta and Gamma ray in dose $1.3158*10^{-8}$ KGy at 2 hr. for Beta ray and $1.973*10^{-10}$ KGy at 3 hr. for Gamma ray; Na^{22}(1Mci) emitte Beta and Gamma ray in dose $1.533*10^{-8}$ KGy at 3hr..The results of exposure showed high improvement production of prodigosin in Americium were 21 isolates (84%), Strontium 17(68%), Cesium 23(92%) and Sodium 12(48%) that give high expression of prodigosin pigment.

Ionizing radiation has many effective uses in medicine of Gamma (γ) radiation with a wavelength minimal than $3x10^{-11}$ meters (greater than 10^{19} Hz and 41.4 keV) (Kwan-Hoong,2003;Weisstein,2014). Gamma radiation can be blocked via a dequetely soild or intense strata of matter, the sealed force of the substance per specified area depends particularly (but not entirely) on the total mass forever the path of the rays(Moulder,2007). Alpha particles react with substance substantially because their charges, combined mass and at their regular velocities only permeate a few centimeters of air or a few millimeters of low intensity substance (such as the thin mica substance which is particularly put in some Geiger counter pipe to permit alpha particles) alpha dissolution do not permit the external layers of dead skin cells and don't occasion deterioration to the live tissues underneath(Dum,2014).

Table(3): Physical mutagenesis of bacterial malignancy tumors by Nd:YAG Lasers.

Nd:YAG		
Pulse	500 pulse	Percentage of killing=87%
Wavelength	1060°A	Viable cells=39 isolates
Time	6 second for each pulse	Control=300 isolates

Results of physical mutagenesis by radiosources in table(3) achieved by Nd:YAG lasers in 500 pulse, the results of mutagenesis exhibit high expression of prodigosin after physical mutagenesis to isolates of *S.marscences* which was 15 isolates (60%) and compared prodigiosin production before the mutagenesis which was 25 isolates (total isolates of *S.marscences*).

A previous study by (Say *et al.,*2015) utilizing long pulsed Nd:YAG laser because the therapy vascular and inflammatory lesions of rosacea. Long-pulsed Nd:YAG laser appear efficacious and secure till the therapy of vascular and inflammatory lesions of rosacea. It may be utilized as first-line therapy in the precocious stages of ETR, it may be concerted with oral/topical antibiotics.

To handle benign prostatic hyperplasia (BPH), Nd:YAG lasers mastery utilized for laser prostate surgery—a compose of transurethral mutilation of the prostate.These lasers are as well utilized broadly in the domain of cosmetic medication for laser hair elimination and the therapy of minor vascular disorder e.g. spider veins on the face and legs. Nd:YAG lasers are as well utilized to therapy Venous Lake lip injury(Azevedo et al.,2010).

Newly utilized for Dissecting cellulitis of the an enemy, a scarce skin disease, Nd:YAG laser utilized for elimination of uterine septa inside of the uterus(Yan *et al.,*2006)

Antibacterial activity of antibiotics of mutant *S.marscences*

The antibacterial effectiveness of antibiotics for mutant *S.marscences* was achieved before the physical mutagenesis and after physical mutagenesis into 33 antibiotics in order that determine the susceptibility of resistance and sensitive as comparsion, results of resistance *S.marscences* before physical mutagenesis improved turn off into sensitive of antibiotics into diverse antibiotics, the results showed that inclusive *E.coli* turn off sensitive ciprofloxacin after physical mutagenesis, as well *A.baumanii* turn off sensitive to Amikacin, Piperacillin; *Morganella morgana*i turn off sensitive to Pipracillin; *K.pneumonia* turn off sensitive to Ciprofloxacin, *Pantoea spp*. turn off sensitive Tetracyclin; *S.aureus* turn off sensitive to Tetracyclin ; *S.epidermidis* turn off sensitive Methicillin and *Streptococcus spp.* turn off sensitive Penicillin G.

Table(4):Antibacterial activity of antibiotics of mutant *S.marscences* after physical mutagenesis.

No.	Bacterial malignant tumors	Turn off sensitive to antibiotics after physical mutagenesis
1	*E.coli*	Ciprofloxacin
2	*A.baumanii*	Amikacin, Piperacilin
3	*Morganella morgani*	Piperacillin
4	*K.pneumonia*	Ciprofloxacin
5	*Pantoea spp.*	Tetracyclin
6	*S.aureus*	Tetracyclin
7	*S.epidermidis*	Methicillin
8	*Streptococcus spp.*	Pencillin G

Antibacterial activity of prodigiosin against bacterial cancer from tumor malignant tumors tissues

Cup disc method or Hole diffusion procedure was utilized to study the restrained impact of prodigiosin product from *Serratia marscences*. The results exhibit the prodigiosin a good inhibitory effectiveness on bacterial cancer(bacterial malignancy tumors) against 28 isolates, results were *S. epidermidis* (0mm), *Salmonella enterica* (18 mm), *E.coli* (19 mm) and *Acinetobacter baumanii* (20 mm) (Bonev *et al.,*2008).

The pigmentation is very changeful of *Serratia* and is reliance on many factors inclusive brood time, and medium component(Kim *et al.,*2007).

The prodigosin secreted outside the bacteria in medium, anothers stay into the cells extracted when cell membrane devastation must that secreted via various procedures. Physical procedure inclusive ultrasonication or chemical procedure such as organic and alcoholic dissolvent that dissolve lipid in the structure of cell membrane as well several solvents utilized to deposition of prodigoisin dye(Nakashima et al.,2005;Song et al.,2006). *S. marcescens* have inimical effectiveness against Gram positive microorganisms and minimal impact against Gram negative bacteri. Prodigiosin described via it is efficient versus a great number of bacteria inclusive against fungi (Antifungal), as well the prodigiosin have high competence versus algae (Algicial factor) and vesus a number of parasites (Kim *et al*.,2007). Prodigiosin has a high medical significance in medication contra tumor cells (Anticancer drug) has expertise and competence against tumor cells inclusive advirse cancer ailment in human breast cancer, leukemia, lung cancer, colon cancer wanting each toxic versus non tumor cells(Soto-Cerrato *et al.,*2007;Wei *et al.,*2010;Dalili *et al.*2011). There are various factors effectiveness production pigment like temperature ,pH , condensation of nitrogen, carbon provenance, condensation of inorganic ions, prong of bacterial increase and lighting sources (Pandey *et al.,*2009) there are various types of *Serratia spp.* according to production of dyye(prodigiosin), pigment production connected with the practicability of bacterial increase and it grows in appropriate brood interval, the second type of *Serratia spp.* called pigment bacteria, another cannot manufacture or formation the pigment except if subsistence of specific amino acid in the environment which

create the pigment, Proline and Alanin(Slater *et al.*,2003;Williamson *et al.*,2007).

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