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Antimicrobial Activity of Bacterial Intracellular and Extracellular Pigment Extract Against Potent Pathogens

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Abstract-

A strain of pigmented *Pseudomonas aeruginosa* was isolated from soil and was analyzed for its pigment's antimicrobial properties. The isolated bacterium was able to produce two types of pigments viz, extracellular and intracellular. The extracellular pigment was green and was water soluble and intracellular pigment was dark red and was not diffusible in water. Both these pigments were extracted and extracted pigments were used for antimicrobial studies. Many potent pathogens were used for this study. Both Gram positive, negative and fungal pathogens were used in this study. The antimicrobial properties were explored by using well diffusion method. MIC values for extracellular pigment against many pathogens were also determined. The extracellular pigment showed notable antimicrobial activity against *C. albicans* ATCC10231 followed by *M. luteus* ATCC9341, *S. aureus* ATCC 6538, *E. coli* ATCC8739 and *P. aeruginosa* ATCC9027. The lowest MIC value was recorded for *C.albicans* (360±00 µg/mL), *E. coli* ATCC8739 (433.3±0.4 µg/mL). The results of this study showed that the bacterium produces extracellular pigment as a protective weapon against many pathogens and competitors on the other hand the intracellular pigment's role is different from extracellular pigment.

Key words- Antimicrobial activity, pathogens, bacterial pigment, MIC

Introduction

Microbes are known to give many valuable bioactive products since long time (Burgess JG et al., 1991; Newman DJ., 2004; Delany, I. et al., 2000; Du L et al., 2007; Larsen et al 2007). Secondary metabolites produced by bacteria have yielded pharmaceutical products (Hamed et al., 2015). Many microbial pigments have medicinal effects like anticarcinogenic effects (Chen et al 1995; Schwartz et al. 1988), antiviral (Sánchez et al.2006), antibacterial (Lichstein et al.1946; Nakamura et al.2003) and antioxidant activities (Konzen et al.2006). Many marine natural

products, especially those isolated from micro-organisms, have already undergone clinical trials (Newman and Cragg, 2004).

The bacteria that do produce pigments has a specific role and effect for the producer organism. If this role and effect is known, one can use this activity and effect for various purposes. The demand for new antibiotics from natural sources and new classes of antibiotics was a major driver in the direction of this research. Investigating the possibility of discovering new antimicrobials from bacteria was selected for proposed research as bacteria have been at war with each other for survival for approximately 4-5 billion years. This always leads one to think that bacteria might be the best source for finding compounds to kill other bacteria.

Materials and Methods

1. Preparation of Pigment Extract- The extracellular pigment was produced in nutrient broth and intracellular pigment was produced on Petri plates containing sterile nutrient agar.

1.1. Intracellular-The intracellular pigment was extracted in ethyl acetate .Equal amount of petroleum ether was added in ethyl acetate solution. The pigment got transferred in petroleum ether layer. The layer was filtered and was dried. The resulting dried pigment was used as extract for all the activities. The extract was abbreviated as IPPE (Intracellular Pink Pigment Extract)

1.2. Extracellular-The green extracellular pigment was extracted from broth by chloroform. Equal amount of chloroform was added in broth in a separatory funnel. The funnel was vigorously shaken and mixture was kept for a minute to separate. The green pigment got transferred into chloroform layer. The chloroform was filtered and filtrate was dried and resulting extract was used in this research study. The extract was abbreviated as EGPE (Extracellular Green Pigment Extract)

2. Determination of antimicrobial properties of extracellular and intracellular pigment-

2.1 Microorganisms used- The antimicrobial activity was evaluated against many pathogens.

The antibacterial activity was determined against both Gram positive and Gram negative bacterial pathogens. The Gram positive pathogens used in this test were *Staphylococci aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 14579, *Bacillus megaterium* ATCC 2326, *Micrococcus luteus* ATCC9341 and the Gram negative bacterial pathogens used were *Escherichia coli* ATCC8739, *Salmonella abony* NCTC6017, *Salmonella typhi* ATCC9207, *Shigella boydii* ATCC 12034, *Enterobacter*

aerogenes ATCC13048, *Klebsiella pneumoniae* ATCC 10031, *Pseudomonas aeruginosa* ATCC9027. *Aspergillus niger* ATCC 16404, *Candida albicans* ATCC10231, *Saccharomyces cerevisiae* ATCC 9763 were used as fungal pathogens.

2.2 Chemicals- For this study Dimethyl sulphoxide (DMSO, ethanol, Chloramphenicol, Fluconazol nutrient agar, Mueller Hinton agar were used.

2.3. Procedure- The antimicrobial activity was evaluated and measured by using agar well diffusion method with minor modifications (Magaldi et. al., 2004). The inoculums of each bacterial and fungal pathogen was developed by growing them overnight in Nutrient broth medium at 37⁰C and then this broth was used for the study. The bacterial suspension was diluted by using sterile saline to adjust the turbidity to the 0.5 McFarland standards. The sterile Mueller Hinton agar plates were inoculated by spreading the 100 µL microbial inoculums of pathogenic bacterium. Agar wells with a diameter of 4-5mm were punched in the inoculated agar plates with a sterile cork borer. 150µL of the pigment extracts with concentration of 1mg/ml were introduced into wells. Chloramphenicol and Fluconazol with the same concentration was used as a positive reference compound. Pigment extracts were prepared in DMSO hence; pure DMSO solution was added in a well to check its activity against pathogenic organisms. These agar plates were incubated at 37⁰C for 24 hours and results were recorded. The formation of zones of inhibition around the wells indicated the antimicrobial activity of the extracts. Gram positive, Gram negative and fungal pathogens were used in this study.

3. Determination of Minimal inhibitory Concentrations(MIC)- Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial (compounds) drug that will inhibit the visible growth of a microorganism after overnight incubation. The MIC was determined for the extracellular pigment only as this pigment showed good antimicrobial result against pathogens. The MIC was determined by following the method and guidelines of Clinical and Laboratory Standard Institute (CLSI).

Result and discussion

The extracellular pigment served very effective and potent antimicrobial agent against all pathogens (Table 1). On the contrary, the intracellular pigment extract (IPPE) showed activity against few pathogens but its activity was comparable against *C. albicans* ATCC10231, a known phytopathogens. It showed very less inhibition activity against Gram positive, Gram negative

pathogen and fungal pathogens other than *C. albicans* ATCC10231. The largest EGPE's zone of inhibition was observed for *C. albicans* ATCC10231 followed by *M. luteus* ATCC9341, *S. aureus* ATCC 6538, *E. coli* ATCC8739 and *P. aeruginosa* ATCC9027; which was $22.6\pm 0.3\text{mm}$, $22.6\pm 0.3\text{mm}$, $21\pm 0.0\text{mm}$, $20\pm 0.5\text{mm}$ respectively as shown in table 1. The EGPE was found to be most effective against *C. albicans* with a lowest MIC value of $360\pm 00\ \mu\text{g/mL}$. The MIC values are shown in Table 2. In Gram negative pathogenic bacterium, the lowest MIC value was obtained for *E. coli* ATCC8739 ($433.3\pm 0.4\ \mu\text{g/mL}$).

Like plant pigments, microbial pigments have specific roles for their protection. If the bacteria is producing very potent compound, this compound could function as drug for human beings also (Lapenda et. al., 2009; Jimtha et. al., 2017) have reported a potent pigment with a good antimicrobial activity. In our study, both the extracted pigments showed a very good antimicrobial activity. The extracellular pigment was found to be active against all types of pathogens (Gram positive, Gram negative and fungal pathogens) and therefore could be referred as broad spectrum antimicrobial. Intracellular pigment was active against only Gram positive pathogens and *C. albicans*. It was not active against Gram negative and other fungal pathogen

Antimicrobial activity																
Antibacterial														Antifungal		
Gram negative pathogens								Gram positive pathogens								
Pathogens	E.Coli ATCC8739	<i>P.aeruginosa</i> ATCC9027	<i>S. abony</i> NCTC6017	<i>E.aerogenes</i>	<i>S. typhi</i> 9207	<i>K.pneumoniae</i> ATCC 10031	<i>M. smegmatis</i> NCIM 607	<i>S. boydii</i> NCIM 9207	<i>B.megaterium</i> ATCC 2326	<i>B.cereus</i> ATCC11788	<i>B.subtilis</i> ATCC 6633	<i>S.aureus</i> ATCC 6538	<i>M.luteus</i> ATCC9341	<i>A.niger</i> ATCC 16404	<i>C.albican</i> ATCC10231	<i>S.cerevisiae</i> ATCC 9763
←Compound																
EGPE	20 ± 0.5	20.1 ± 2.2	14 ± 00	17.3 ± 0.3	15.3 ± 0.3	11.6 ± 0.3	15 ± 00	15.6 ± 0.3	20.1 ± 1.1	19.6 ± 0.3	18 ± 0.3	21 ± 00	22.6 ± 0.3	17.3 ± 0.3	22.6 ± 0.3	18±0.5
IPPE	7.0 ± 0.0	-	-	10 ± 0.5	-	-	-	9 ± 0.3	-	10.6 ± 0.3	11.3 ± 0.3	-	10.6 ± 0.3	-	12.5 ± 0.2	-
Chloramphenicol/Nystatin	28 ± 0.5	-	32 ± 0.5	26.3 ± 0.3	-	27.6 ± 0.3	28.3 ± 0.3	31.3 ± 0.3	-	27.6 ± 0.3	29 ± 00	30.3 ± 0.3	29.6 ± 0.3	30.6 ± 0.3	30.3 ± 0.3	28±0.5
DMSO	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0 0

Table 1-Antimicrobial activity of EGPE(Extracellular green pigment extract) and IPPE(Intracellular Pink Pigment extract). The results are the mean ± SD

Pathogens used	<i>E. Coli</i> ATCC8739	<i>E. aerogenes</i>	<i>P. aeruginosa</i> ATCC9027	<i>S. typhi</i> 9207	<i>K. pneumoniae</i> 10031	<i>M. smegmatis</i> NCIM 607	<i>S. boydii</i> NCIM 9207	<i>S. abony</i> NCTC6017	<i>B. cereus</i> ATCC11778	<i>B. subtilis</i> ATCC 6633	<i>S. aureus</i> ATCC 6538	<i>A. niger</i> ATCC 16404	<i>C. albican</i> ATCC10231	<i>S. cerevisiae</i> ATCC 9763
MIC value(μ g/ml) of EGPE	433.3 ± 0.4	560 ± 5.7	450 ± 5.7	616.6 ± 3.3	666.6 ± 3.3	613.3 ± 3.3	600 ± 0.0	590 ± 5.7	460 ± 5.7	523.3 ± 3.3	413.3 ± 3.3	573.3 ± 3.3	360 ± 00	566.6 ± 3.3

Table 2- MIC values of EGPE (Extracellular green pigment extract) against pathogens. The results are the mean \pm SD.

References-

- Burgess JG, Hideaki M, Hiroaki S, Tadashi M. Antibiotic production by the marine photosynthetic bacterium *Chromatium purpuratum* NKPB 031704: localization of activity to the chromatophores. FEMS Microbiology Letters, 1991; 84(3): 301–305.
- Chen F, Zhang Q. Inhibitive effects of *Spirulina* on aberrant crypts in colon induced by dimethyl hydrazine. Zhonghua Yu Fang Yi Xue Za Zhi, 1995: 29:70.
- Delany I, Sheehan MM, Fenton A., Bardin S, Aarons S, O’Gara F. Regulation of production of the antifungal metabolite 2,4-diacetylphloroglucinol in *Pseudomonas fluorescens* F113: genetic analysis of phlF as a transcriptional repressor. Microbiology, 2000; 146: 537–546.
- Du L, Zhu T, Fang Y, Liu H, Gu Q, Zhu W. Aspergiolide A, a novel anthraquinone derivative with naphtho[1,2,3-de]chromene-2,7-dione skeleton isolated from a marine-derived fungus *Aspergillus glaucus*. Tetrahedron, 2007; 63: 1085–1088.
- Hamed SR, Mohamed SS, Raed SA, Selim MS. Production of Secondary Metabolites as Antioxidants from Marine-Derived Fungi and Bacteria. International Journal of ChemTech Research, 2015; 8:92-99.
- Jimtha JC, Jishma P, Sreelekha S, Chithra S, Radhakrishnan EK. Antifungal properties of prodigiosin producing rhizospheric *Serratia spp.* 2017.DOI: <http://dx.doi.org/10.1016/j.rhisph.2017.02.003>.
- Konzen M, De Marco D, Cordova CA, Vieira TO, Antônio RV, Creczynski-Pasa TB. Antioxidant properties of violacein: possible relation on its biological function. Bioorg Med Chem., 2006, 14:8307-13.
- Lapenda JC, Silva PA, Vicalvi MC, Sena KXFR, Nascimento SC. Antimicrobial activity of prodigiosin isolated from *Serratia marcescens* UFPEA 398. Food Chemistry, 2009. 117: 731–738.

9. Larsen TO, Lange L, Schnorr K, Stender S, Frisvad JC. Solistatinol, a novel phenolic compactin analogue from *Penicillium solitum*. *Tetrahedron Lett*, 2007; 48: 1261–1264.
10. Magaldi S, Mata-Essayag S, Hartung de C. C. Well diffusion for antifungal susceptibility testing. *Int. J. Infect. Dis.*, 2004; 8: 39-45.
11. Newman DJ, Cragg GM. Marine natural products and related compounds in clinical and advanced preclinical trials. *J Nat Prod*, 2004; 67: 1216–1238.
12. Sánchez C, Brana AF, Méndez C, Salas JA. Reevaluation of the violacein biosynthetic pathway and its relationship to indolocarbazole biosynthesis. *Chem Bio Chem*, 2006; 7:1231-40.
13. Lichstein HC, Van De Sand VF (1946) The antibiotic activity of violacein, prodigiosin, and phthiocoumarin. *J Bacteriol* 52 :145–6.