Advances in Bioresearch
Adv. Biores., Vol 15 (6) November 2024: 169-176
©2024 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.15.6.169176

Advances in Bioresearch

ORIGINAL ARTICLE

Biomedical Application of Microbial Pigment Extracted from *Hallobacterium* Sp Isolated from Marine Water

Jamith Basha Abdul Wahid[1*]
Medical laboratory, Faculty of Applied Medical Sciences, Northern Border University, Saudi Arabia.
*Corresponding Author's Email: jamithbasha@gmail.com

ABSTRACT
The primary objective of the study is to isolate pigment producing marine bacteria from seawater. Conventional enrichment isolation was used. The antioxidant by DPPH, anti-inflammatory by HRBC, albumin denaturation and antibacterial was performed followed by pigment production. The selective enrichment isolation has one salt tolerant pigmented producing colony have been selected and identified by biochemical characters. The pigment producer identified as gram-negative rod, salt tolerant, Indole, MR and catalase positive. Pigmentation on Chemically defined media reveal that tryptic soy agar and nutrient agar influenced significant growth and intracellular pigment higher yield. Under submerged TS broth the pH 5-6 supports the growth and synthesis of pigments on liquid medium. Strain grown on tryptic soy agar had maximum intracellular pigment. Extracellular pigment production is influenced by pH of medium. Among the different pH, production of pigment found between 5 and 6 has concentrated pigmentation. Concentration dependent antibacterial, antioxidant, anti-inflammatory activity of pigment was recorded. The pigment FTIR reflects predigosin like characters. The pigment had significant free radical scavenging and anti-inflammatory effect equal to standard drugs. The findings conclude that the Halobacterium sp produced pigment have potent medicinal value used as antibacterial, antioxidant and anti-inflammatory agent followed by toxicity evaluation.
Keywords: Hallophiles, antioxidant, anticancer, inflammation, predigosin

Received 24.08.2024 Revised 01.09.2024 Accepted 11.11.2024

How to cite this article:
Jamith Basha Abdul Wahid. Biomedical Application of Microbial Pigment Extracted from *Hallobacterium* Sp Isolated from Marine Water. Adv. Biores., Vol 15 (6) November 2024: 169-176.

INTRODUCTION

Research on extremophiles are particularly crucial for enhancing the extraction of novel chemicals and their potential uses in biomedicine. The halophilic bacteria are thought to produce hydrolytic substances, carotenoids, retina protein molecules, and viable solutes as stabilizers of macromolecules, biological polymers, and fertilizer (4). In general, halophilic microorganisms thrive in varying NaCl centralizations ranging from 2 to 30%. With 129 animal species, Halobacteriaceae is the largest category of halophilic Archaea and contains the strains that require the most salt. The distinctive feature of a hypersaline atmosphere is that, aside from high salt concentrations, it allows for an exceptional variety of microorganisms to live there (21). Halobacteriaceae helps produce certain carotenoid colors that aid in light absorption and increasing disappearing in saltern crystallizer ponds (7). Bacteriorhodopsin, a vital film protein produced by *Halobacterium salinarum*, is used as holographic stockpile material in PC memory and preparation units. A vast variety of halophiles have been employed and studied for their distinct biotechnological uses in the food sector as compounds of coloring and cryoprotectants. At the industrial level, halophilic microorganisms have successfully provided salt-tolerant exoenzymes, such as lipases, amylases, and proteases, to numerous compounds used in the restorative industry (9). The minimal natural impact caused by microbial pigment during synthesis is one of its main advantages. Additionally, normal colors enhance an item's aesthetic appeal and provide extra advantages including cellular strengthening and antibacterial, antioxidant qualities (20). In order to prevent oxidative damage in stomach ulcers, *Pseudoaltero monas* and *Chromobacter violaceum* produce Violacein , an incredible cell reinforcement that animates mucosal defense components. Lutein, a substance that prevents cancer, is

produced by *Spongiococcum excentricum*. *Monascus* sp. demonstrated the production of monascorubramin (red) and antibacterial anthraquinoid. Many studies have taken into account the antioxidant riboflavin produced by *Ashbya gossypii* and the antibacterial lycopene (red) *Blakesle atrispora* (22). *Micrococcus luteus* -derived pigments demonstrated encouraging antibacterial efficacy towards organisms responsible for wounds (23). *Pseudoalteromonas* sp. produce prodigiosin is one kind of pigment that is lethal to leukemia cells (25).Many microorganisms provide various hues that eventually satisfy the demands of contemporary applications. The goal of this study is to identify and assess the potential of extremophilic pigment-producing microorganisms.

MATERIAL AND METHODS

Isolation of bacteria: Bacterial strains are isolated by enrichment method. Double strength of tryptic soy (TS) broth enriched with 10% of sodium chloride was prepared and autoclaved. Sterile broth inoculated with 1mL of red sea water sample, Saudi Arabia and kept incubation for 24-48 h. enriched sample diluted up to 10^7 and one ml of final dilution is plated on modified nutrient agar. TSA with 10 % NaCl was prepared and used for pour plate technique. Plates were incubated for 24 h. pigment producing bacteria grown on the medium selected and characterized. Biochemical characters like Gram's staining, IMViC, catalase and oxidase were performed.

Solid state pigment production: Nutrient agar, minimal agar, tryptic soy agar plates were prepared and lawn culture of strain were prepared. Followed by 48h incubation 25 ml of ethanol was added and pigment was excluded .weight of cells calculated followed by taken of pre weight agar plate. The mixture was vigorously vortexed for 2 min. The solution was then centrifuged for 10 min at 6000 rpm and eluted with silica 120 mesh column. The OD of eluted pigment taken at 600 nm.

OD x (dilution factor) x (total volume of pigment) / Weight of cell

Effect of medium pH on pigment production (18)

TS broth was prepared at different pH (2-10) by adjusting medium pH with 1N HCL and 1N NaOH then autoclaved at 121ºC for 15 min, cooled, inoculated with 1 mL of culture of bacterium and incubated at 37°C for 48h.pigment were extracted broth after centrifugation at 7,000 rpm with ethanol and vaccum dried. The dried pigment redissolved in 10 ml ethanol. The OD of eluted pigment taken at 600 nm.

OD x (dilution factor) x (total volume of pigment) / Weight of pigment extracted

Characterization of pigment

Extracted pigment purified with silica column with ethanol-acetone solvent and purity detected by TLC using chloroform acetone and methanol as mobile phase (5:2:1). The nature of pigment subjected to FTIR characterization

Antibacterial activity

Screening of antimicrobial activity of crude pigment was determined by disc diffusion method at 25-100 µg/disc. 24h old *P.aeruginosa* bacterium was spread over on MH Petri agar plate under aseptic conditions. Known concentration of crude pigment loaded on sterile disc at different concentration. All the concentration was kept in triplicate manner. Acetone and ethanol alone is applied as negative control. Ofloxacin used as positive control. The entire disc placed over agar preloaded with test pathogen. The plates are kept incubation at 37°C for 24 hours. After incubation for 24 hours at 37°C the zone of inhibition was measured with the help of standard scale.

Synergy test

The stock solutions and twofold serial dilutions of each drug to at least double the MIC were prepared according to NCCLS. Columns 1 to 7 contain 2-fold serial dilutions of ofloxacin, and rows A to G contain 2-fold serial dilutions of pigment. One column contains a serial dilution of pigment alone, while one row contains a serial dilution of ofloxacin alone. about 200 µl of Mueller-Hinton broth, ofloxacin 200ul (200µG) was serially diluted to get 100, 50, 25, 12.5, 6.25, 3.125 and 1.5µg, while the second drug pigment was added at different concentration to the rows. An inoculum equal to a 0.5 McFarland turbidity standard was prepared from *P.aeruginosa*. plates was inoculated with 100 µl of a bacterial inoculum of 5 × 10^5 CFU/ml, and the plates were incubated at 35°C for 48 h under aerobic conditions. The resulting checkerboard contains each combination of two drugs antibiotic and pigment, contain the highest concentration of each antibiotic at opposite corners. Synergy is more likely to be expressed when the ratio of the concentration of each antibiotic to the MIC of that antibiotic was same for all components of the mixture. The combination is considered synergistic when the ΣFIC is ≤0.5, indifferent when the ΣFIC is >0.5 to <2, and antagonistic when the ΣFIC is ≥2.

- The ΣFICs were calculated as follows: ΣFIC = FIC A + FIC B,
- where FIC A is the MIC of drug A in the combination/MIC of drug A alone,
- and FIC B is the MIC of drug B in the combination/MIC of drug B alone.

Antioxidant activity

1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay on intra and extracellular pigment at 5mg/ mL was taken to determine antioxidant activity. The standards used in this test were ascorbic acid at 100 µg concentration. The final concentration pigment used in the study was 50 and 100 µg/ml. 2 mL of ethanol solution was taken in a test tube and 0.2 ml of DPPH reagent was mixed. Sample/standard 0.2mL was added. Then, the tube was wrapped in aluminum foil and incubated on the shaker at room temperature for 20 minutes. The absorbance of control(C) and test(T) was recorded at 517 nm.

C-T/C X 100

HRBC anti-inflammatory activity

1mL of blood was diluted with 20 ml of PBS solution. This HRBC suspension was used for the estimation of anti-inflammatory property. Different concentrations of pigment, standard *Diclofenac* and control were separately mixed with 1mL of phosphate buffer, 2 mL of hyposaline (0.36% NaCl) and 0.5 mL of HRBC suspension. All the assay mixtures were incubated at 37 °C for 30 minutes and centrifuged at 3000 rpm. The supernatant liquid was decanted and the hemoglobin content was estimated by a spectrophotometer at 560 nm. The percentage of membrane protection was estimated by assuming the hemolysis produced in the control as 100%.

Percentage protection= 100- (OD sample/ OD control) × 100

The anti-inflammatory protein denaturation

Anti-inflammatory activity of pigment was studied by using inhibition of protein denaturation method. The reaction mixture (5ml) consist of 0.2 ml of egg albumin (hen's egg), 2.8 ml phosphate buffered saline (pH: 6.4) and 2 ml of varying concentration of plant extracts. Similar volume of double distilled water served as control. Then the mixtures were incubated at 37±2 °C in an incubator for 15 minutes and then heated at 70 ºC for 5 minutes. After cooling, their absorbance was measured at 660nm by using vehicle as blank. Diclofenac standard was used as reference drug and treated similarly for determination of absorbance. The Percentage inhibition of protein denaturation was calculated as follows

ODC-ODS/ODC X 100

RESULTS AND DISCUSSION

Isolation of pigment producing bacteria

The organism under study was Pinkish orange pigmented salt loving bacterium which is gram negative, rod, catalyst positive, oxidase negative. Totally 13X 10^7 colonies forming unit were recorded on enriched agar plate method. majority of colonies are opaque white-coloured colonies and few are pigmented. colonies developed with, pigmented selected and subcultured on TS agar (Fig 1). The organism under study was Pinkish orange pigmented salt loving bacterium biochemically characterized and data given in table 1. The isolates was gram negative rod, indole, MR, catalase positive, negative on citrate, VP and oxidase. The biochemical features of isolates reveals that isolate belongs to *Halobacterium sp*. study based on extremely halophilic capable of producing red pigments known as carotenoids which gives red, yellow and orange colour to the organism and also to compact the high salt and intense UV radiation by violacein producers (26). Previously *Halobacterium salinarum* from saline lakes, magrooves and salt sediments were isolated are reported to produce pigments(11)

Characterization of pigment

TLC analysis of pure pigments reveals presence of single fraction on both intra and extracellular The pigment of crude and purified reveals both crude and purified shows 0.38 R_f and no changes were observed in retention factor. The FTIR spectrum of column pured pigment is given in Figure 2. Data reveal that the extracted pigment have medium vibration at 3405 cm^{-1}, 2,995 cm^{-1} related to N-H stretch and O-H stretch of carboxylic acid. A weak Peak at 2090 cm^{-1} related to C≡C alkyl. Absorption at 1770-1759 and 1637 cm^{-1} corresponding to C=O stretching and C=C stretching of imine / oxime group. Furthermore the weak absorption at 1454 cm^{-1} indicated C-F stretching, and 1382-1357 cm^{-1} showed the S=O stretch, while the peak 930 and 711 cm^{-1} indicated the presence of C-O-C and C-H phenyl ring bend. All the N-H group and phenyl rings were recorded are fingerprint region, which was characterized by the weak intensity. Previous data on prodigiosin exhibits similar absorptions between 1600-800 cm^{-1} reported by Lazović et al. (15). Prodigiosin is a kind of pigment synthesized by different genera including Bacillus sp, Streptomyces and *Serratia marcescens.* presence of COOH functional group in pigments reported earlier by Fengwei *et al* (8). Anticancer Prodigiosin pigment from *S.marcescens* have similar functional groups (14). Presence of C-O-C (900 cm^{-1}) was correlate with the report of zeaxanthin as reported by Hizbullahi Usman *et al*. (12)

Extraction and quantification of pigment

The pigment from isolate grown on nutrient agar, Minimal agar and TS agar were separated by solvent extraction and purified with column chromatography. The figure 3a represent the yield of intracellular pigment at unit level shows maximum pigmentation among three different medium. Maximum production 546±1.73 U in TS medium followed by nutrient agar (354.66± 3.05U). Less significant pigmentation 141±0.69 U was produced under minimal agar. Likewise the quantity of pigment from submerged broth was found best at neutral range and less significant among acidic pH and extreme alkaline pH (Figure 3B). at 5 and 6 pH the growth and pigmentation was not affected but showed variation on pigmentation. The unit of EC pigment was 4.7±0.1, 85.76±2.89, 92.46±0.41respectively at pH 4, 5, 6 and no pigmentation was recorded at pH 2 and 8. Pigment producing bacteria capable to grow well on pH of neutral range was earlier reported in many studies (3). Further similar report on production of pigment on submerged sate was influenced by biomass of cells regulated by pH of medium (10).

Antibacterial activity of pigment

The results of pigment from intracellular and extracellular pigment against ofloxacin resistant *P.aeruginosa* implies that the higher concentration of intracellular pigment have had good antibacterial at 100 µL. The antibacterial activity of Intracellular Pigment was found to be concentration dependent. The zone of inhibition were 12.66±1.15, 15.3±1.15, 16.33±0.57 and 18.3±0.57 mm respectively among 25, 50,75 and 100µg. compare to intracellular the extracellular pigment activity was moderate and recorded as 7.33±1.15, 11.3±1.15, 14.66±0.57 and 16.3±0.57. The inhibition of ofloaxacin was less significant at 25 to 50 compare to pigment but significant at 50-100ug. The zone size of standard among the tested concentration was 6±0, 10.6±1.15, 15.33±1.15 and 16±1 mm. The t-test of intracellular and standard reveals that the $p<0.05$ of pigment was significant at 25, 50 and 100µg and not significant at 75µg($p>0.05$). likewise extracellular pigment showed $p>0.05$ found to be not significant (table 2). In addition microbial pigment has synergy effect with amoxicillin and activity against MDR *P.aeruginosa* was given in table 3. Further the MIC of tested pigment is estimated as 100 µg and the MIC of amoxicillin was 50µg against *P.aeruginosa.* The checkerboard assay reveals have given fractional inhibitory 0.25 and 0.125 respectively for pigment and antibiotic have given 0.37 ΣFICs denotes that both having synergistic effect (Table 3). Activity of many pigments against gram-negative bacteria depends on yield of pigment due to the permeability of cells, whereas gram positive are does not require high concentration of prodigiosin. Pigments from bacteria proven to be good antifungal, antibacterial (2), antiprotozoal, antimalarial, cytotoxic and immunosuppressive activities (17). The pigment showed good antibacterial activity showed maximum zone of inhibition 16 mm against *P.aeruginosa.*

Anti inflammatory assay

In vitro anti-inflammatory activity of formulated pigment gel evaluated using albumin denaturation and membrane stabilization assay. *Diclofenac* was used as a standard drug for the study of anti-inflammatory activity. Pigment showed anti-inflammatory activity by inhibiting the temperature elevated albumin coagulation 95% at 500 µg/mL and minimum inhibition rate 47% at 62.5µg/ml (table 4). By increasing concentration of pigment from 62.5 to 500 the albumin denaturation was inhibited greatly. The concentration dependent percentage of inhibition of pigment were 47±1, 52.66±0.57, and 71±195.33±0.57 percentage with IC50 of 281 and for standard were 84.33±0.57, 87.33±0.57, 89.33±0.57 and 92.33±0.57% with IC50 of 178.25. The p value is significant ($p<0.05$) at 250 and 500µg and the data is not significant at 62.5 and 125µg. Likewise HRBC assay on red blood cells membrane stabilization of standard and sample given in Table 5. Data reveals that pigment have anti-inflammatory potential close to standard. Pigment based gel showed concentration dependent RBC membrane stabilization activity. At higher concentration hemolysis was increased in pigment. The percentage of membrane stabilization were 74.33±0.57, 79.33±0.57, 80.66±0.57 and 84.33±0.57 respectively at 500, 250,125 and 62.5 µg/mL. The activity of Diclofenac was 82.33±0.57,84.33±0.57,88.66±0.57, 92.66±0.57. The independent t test were significant at higher concentration 25-500 and not significant at 62.5-125µg. The HRBC data confirm increases in concentration decreases stabilization of RBC membrane in test as well as standard (table 3). Orange pigment derivatives from Monascus have reported as good anti-inflammatory activities by Choe et al (6). Pigments like pink, yellow, orange, and brown from marine *Brevibacterium* sp with effective anti-inflammatory activity was reported by Srilekha et al (18). Bacterial pigments are reported as alternative biomedicine can be used for may biomedical application (1)

Antioxidant assay

DPPH antioxidant of extracellular, intracellular pigment and standard ascorbic acid have given promising antioxidant potential and the percentage free radical scavenging property was given in figure 4. LB broth extracted pigment showed LESS antioxidant activity than intracellular pigment. the activity IC pigment

was close to the activity of standard ascorbic acid. Percentage of IC pigment free radical inhibition was 35.33±1.15 and 47.66±0.90. EC pigment have 54.66±1.15 and 68±1 percentage DPPH inhibition. Standard showed 47.333±2.081 and 70.66±1.15 respectively at 50 µg and 100 µg. In vitro study using carotenoid pigment extracted from the *Sporobolomyces* sp tested and exhibited antioxidant and antimicrobial property reported by Manimala and Murugesan (16). Kajal et al (13) evaluated microbial pigment antioxidant nature and reported concentration free radical scavenging activity. Another study conducted by Turki et al (22) reported antibacterial pigment from Bacillus strains have potent ABTS scavenging assay with low SC_{50} .

Table 1. Biochemical characteristic of bacterial isolate

TEST	RESULT
Grams stain	- rod
Oxidase	Negative
Catalase	Positive
Indole	Positive
MR	Positive
VP	Negative
Citrate	Negative

Table 2. Zone of inhibition mm in diameter

SAMPLE	25µg	P	50 µg	P	75 µg	P	100 µg	P
Intracellular Pigment	12.6±1.15	0.0049	15.3±1.15	0.0099	16.33±0.57	0.11	18.3±0.57	0.036
Extracellular Pigment	7.33±1.15	0.0917	11.3±1.15	0.2113	14.66±0.57	0.264	16.3±0.57	0.333
Oflaxacin	6±0		10.6±1.15		15.33±1.15		16±1	

Table 3 MIC and FIC of Intracellular pigment

CONC. OF AMOXICILLIN			CON OF PIGMENT			
	CONTROL		12.5	25	50	100
			+	+	+	-
	100	+	-	+	-	-
	50	-	-	+	-	-
	25	+	+	+	-	-
	12.5	+	-	+	-	-

Table 4. Percentage of anti inflammatory activity of pigment

Concentration of pigment	Inhibition of Albumin denaturation	P value
62.5	47±1	2.47948E-05
125	52.66±0.57	4.62214E-05
250	71±1	0.000165207
500	95.33±0.57	0.001562795
IC50	281.6µG	
Concentration of Diclofenac		
62.5	84.33±0.57	-
125	87.33±0.57	-
250	89.33±0.57	-
500	92.33±0.57	-
IC50	178.25 µG	

Table 5. Percentage of membrane stabilization on HRBC

Concentration µg/mL	Pigment	*Diclofenac*	P value
62.5	84.33±0.57	92.66±0.57	1.305
125	80.66±0.57	88.66±0.57	1.919
250	79.33±0.57	84.33±0.57	0.0007
500	74.33±0.57	82.33±0.57	0.006

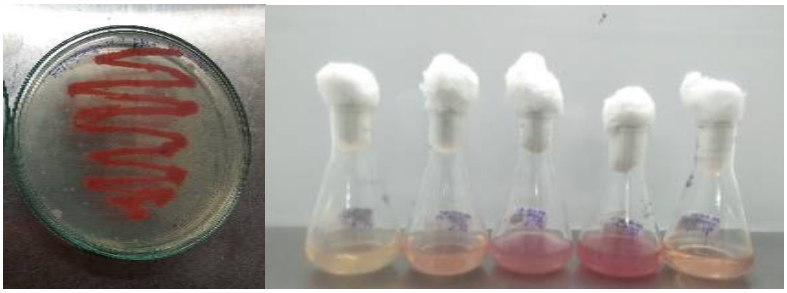

Figure 1. Isolated halobacterium and pigment production

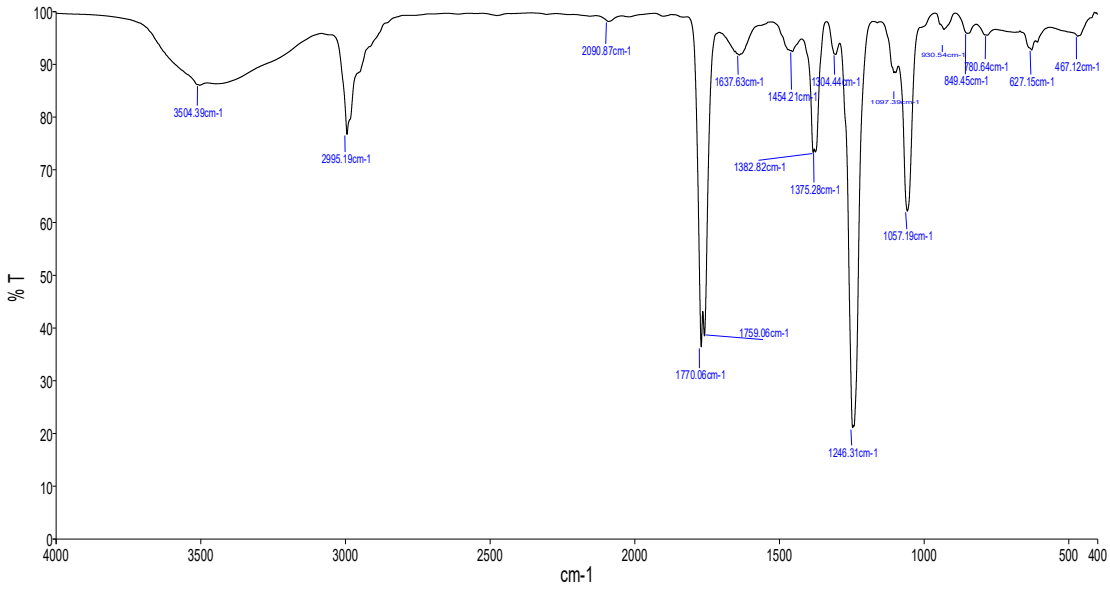

Figure 2. FTIR spectrum of pure pigment

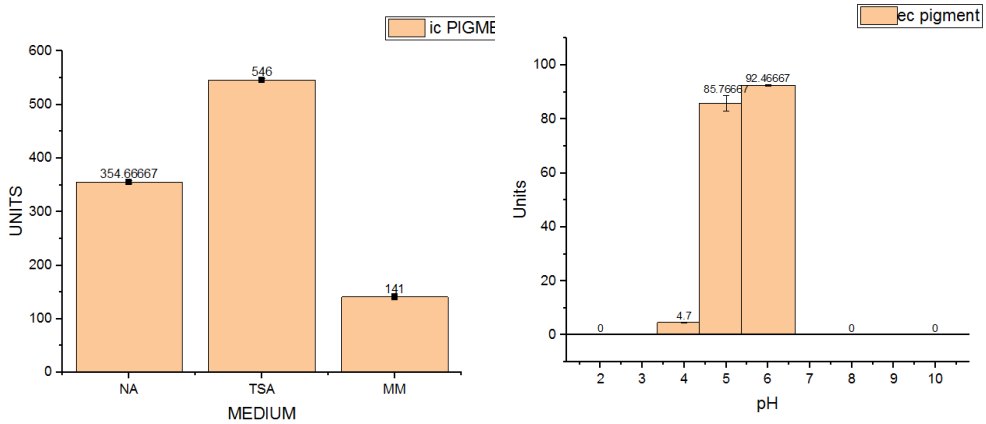

Figure 3. Yield of pigment a) Intracellular b)Extracellular

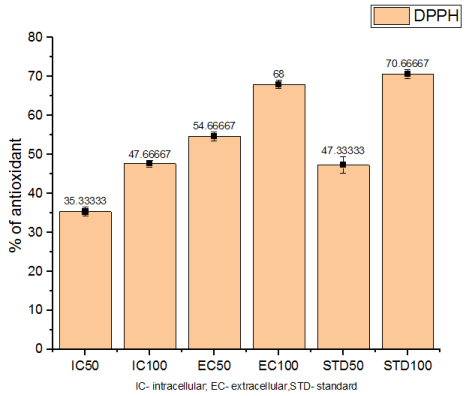

Figure 4. Antioxidant activity of pigment

CONCLUSION

The reddish pink pigment producing bacteria isolated from seawater identified as *Hallobacterium* sp. The intracellular and extracellular pigment extracted, column purified and found to be antibacterial, antioxidant and anti-inflammatory in nature. Besides antibacterial, it also showed synergistic properties. The antioxidant nature of pigment may help to inhibit the onset of cancer by prevent free radicals. The significant influence genetics of isolated *Halobacterium* sp should be investigated in future studies.

ACKNOWLEDGEMENT

The authors extend their appreciation to the Deanship of Scientific Research at Northern Border University, Arar, KSA for funding this research work through the project number "NBU-FFR-2024-1329-04".

REFERENCES

1. Adzzie Shazleen. Azmana., Christina-Injan, Mawang.,& Sazaly, Abubakar (2018). Bacterial Pigments: The Bioactivities and as an Alternative for Therapeutic Applications. Natural Product Communications, 13(12),1747-1754.
2. Ajay kumar., Usha verma., & Heena, S (2012). Antibacterial Activity *Monascus purpureus* (Red pigment) Isolated from Rice Malt. Asian Journal of Biological and Life Sciences,1(3), 252-255.
3. Alejandro Méndez., Catalina Pérez., Julio Cesar Montañéz., Gabriela Martínez and Cristóbal Noé Aguilar (2011).Red pigment production by *Penicillium purpurogenum* GH2 is influenced by pH and temperature. Journal of Zhejiang University Science B.12;961–968.
4. Amoozegar, MA., Siroosi, M., Atashgahi, S., Smidt, H., Ventosa, A (2017). Systematics of haloarchaea and biotechnological potential of their hydrolytic enzymes. Microbiology, 163(5),623-645
5. Banerjee, D., Mondal, A., Gupta, M., Guha, A. K., and Ray, L. (2013). Optimization of fermentation conditions for green pigment production from *Bacillus cereus* M116 (MTCC 5521) and its pharmacological application. Lett. Appl. Microbiol, 58, 25–30
6. Choe, D., Song, SM., Shin, CS (2020) Production and Characterization of Anti-Inflammatory Monascus Pigment Derivatives. Foods,9(7),858.
7. De la Haba, RR., Sanchez-Porro, C., Marquez, MC., Ventosa, A. (2010). Taxonomic study of the genus Salinicola: transfer of *Halomonassalaria* and *Chromohalobacter salarius* to the genus Salinicola as *Salinicola salaries* comb. nov.and *Salinicola halophilus* nom. nov., respectively. Int. J. Syst. Evol. Microbiol, 60.
8. Fengwei, Li., Feng, Xue.,& Xiao Hong, Yu (2017). GC–MS, FTIR and Raman Analysis of Antioxidant Components of Red Pigments from Stemphylium lycopersici. Current Microbiology, 74, 532–539.
9. Ghasemi, Y., Rasoul-Amini, S., Ebrahiminezhad, A. (2011).Screening and Isolation of Extracellular Protease Producing Bacteria from the Maharloo Salt Lake. Iran. J. Pharm. Sci, 7,175-180
10. Haddix, P.L., & Shanks, R.M.Q. (2018). Prodigiosin pigment of *Serratia marcescens* is associated with increased biomass production. Archives of Microbiology, 200(7), 989–999.
11. Hassanshahian, M., & Mohamadian, Jafar (2011) Isolation and characterization of *Halobacterium salinarum* from saline lakes in Iran. Jundishapur Journal of Microbiology, 4 (2), S59-S65.
12. Hizbullahi Usman, M., Farouq, AA., Baki, AS., Abdulkadir, N., & Mustapha, G (2018) Production and characterization of orange pigment produced by Halophilic bacterium *Salinococcus roseus* isolated from Abattoir soil. J Microbiol Exp, 6, 6:238.
13. Kajal Satpute., Arti Kale., & Rajesh Sharma (2020) Assessment of Anti oxidation potential, Lipid peroxidation, and cytotoxicity of microbial pigment. International Journal of Scientific & Engineering Research, 11(1), 709-800,
14. Lazic, J., Skaro Bogojevic, S., Vojnovic, S., Aleksic, I., Milivojevic, D., Kretzschmar, M., Gulder, T., Petkovic, M., Nikodinovic-Runic, J. (2022) Synthesis, Anticancer Potential and Comprehensive Toxicity Studies of Novel

Brominated Derivatives of Bacterial Biopigment Prodigiosin from Serratia marcescens ATCC 27117. Molecules, 27(12), 3729.
15. Lazović, S., Leskovac, A., Petrovic, S., Senerovic, L., Krivokapic, N., Mitrovic, T., Bozovic, N., Vasic, V., & Nikodinovic-Runic, J. (2017). Biological effects of bacterial pigment undecylprodigiosin on human blood cells treated with atmospheric gas plasma in vitro Experimental and Toxicologic Pathology, 69(1), 55-62.
16. Manimala, M.R.A., & Murugesan, R. (2014) In vitro antioxidant and antimicrobial activity of carotenoid pigment extracted from *Sporobolomyces* sp. isolated from natural source. Journal of Applied and Natural Science, 6(2), 649-653.
17. Palacio-Castañeda, V., Pérez-Hoyos, Alejandra., Carrascal-Correa, Daniel., Osorio-Echeverri., Víctor, Manuel (2019) Antibacterial pigment production by *Serratia marcescens* using different casein types obtained from milk. Rev. Colomb. Biotecnol, 21(1), 82-90
18. Samrot, AV., Chandana, K., Senthilkumar, P., Narendra, K (2011) Optimization of prodigiosin production by *Serratia marcescens* SU-10 and evaluation of its bioactivity. Int Res J Biotechnol, 2,128–133
19. Srilekha, V., Krishna, G., Seshasrinivas, V., Charya, MAS. (2017) Antibacterial and anti-inflammatory activities of marine Brevibacterium sp. Res Pharm Sci, 12(4), 283-289
20. Suryawanshi, RK., Patil, CD., Borase, HP., Salunke, BK., Patil, SV. (2014) Studies on production and biological potential of prodigiosin by *Serratia marcescens*. Appl Biochem Biotechnol, 173(5),1209-21
21. Tseng, Y.Y. Chen, M.T., & Lin C.F. (2000). Growth, pigment production and protease activity of Monascus purpureus as affected by salt, sodium nitrite, polyphosphate and various sugars. Journal of Applied Microbiology.88(1), 31-37.
22. Tuli, HS., Chaudhary, P., Beniwal, V., Sharma, AK (2015) Microbial pigments as natural color sources: current trends and future perspectives. J Food SciTechnol, 52(8),4669–4678
23. Turki, M Dawoud., Alharbi, NS., Theruvinthalakal, AM., Thekkangil, A., Kadaikunnan, S., Khaled, JM., Almanaa, TN., Sankar, K., Innasimuthu, GM., Alanzi, KF., Rajaram, SK. (2020) Characterization and antifungal activity of the yellow pigment produced by a Bacillus sp. DBS4 isolated from the lichen *Dirinaria agealita*. Saudi J Biol Sci, 27(5),1403-1411
24. Umadevi, K., & Krishnaveni, M. (2013). Antibacterial activity of pigment produced from *Micrococcus luteus* KF532949. Int. J. Chem. Anal. Sci, 4, 149–152
25. Wang, Y., Nakajima, A., Hosokawa, K., Soliev, A.B., Osaka, I., Arakawa, R., et al. (2012). Cytotoxic prodigiosin family pigments from *Pseudoalteromonas* sp. 1020R isolated from the Pacific coast of Japan. Biosci. Biotechnol. Biochem, 76, 1229–1232
26. Yada, S., Wang, Y., Zou, Y., Nagasaki, K., Hosokawa, K., Osaka, I., et al. (2008). Isolation and characterization of two groups of novel marine bacteria producing violacein. Mar. Biotechnol,10, 128–132