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Antibacterial Activity of Pigment Producing Secondary Metabolites from Halophilic Bacteria

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ABSTRACT
This study aimed to extracted the pigmented secondary metabolites produced by halophilic bacteria isolated from marakanam saltpan soil samples. Extracted pigmented secondary metabolites were screened for their antibacterial activity against Staphylococcus aureus, Streptococcus pyogens, Streptococcus pneumoniae, Escherichia coli, klebsiella pneumoniae, Serratia marcescens bacterial pathogens. Ethyl acetate extract of light orange color pigmented secondary metabolites from MSP8 isolates showing good antibacterial activity against all pathogens. MSP8 Pigmented ethyl acetate extract were characterized by FT-IR and GC-MS analysis. GC-Ms analysis results revealed 1,2,2-Trimethylcyclopropropylamine with retention time 5.992, 1,2-Propadiene (CAS) Allene with retention time 7.853, trans-beta-ionon-5,6-epoxide 8.560compounds were detected.
Keywords: Bacterial pathogens, Pigments, Secondary metabolites, FT-IR and GC-MS analysis.

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INTRODUCTION

Hypersaline environments have very high salinity much compare than seawater. Hypersaline environments include hypersaline lakes, deep ocean brine pools, industrial effluents and solar salterns. Solar salterns are one of the extreme environments with high NaCl concentration which are used for commercial salt production. Ancient salt production technology involves concentration of seawater by allowing it to pass through a series of interconnected ponds[1]. Halophiles are a group of microorganisms that includes bacteria, archaea and fungi. The halophiles are classified into three, based on their salt tolerance: viz, slight halophiles (2-5% NaCl), moderate halophiles (5-20% NaCl) and extreme halophiles (20-30%) [2]. The halophilic microorganisms differ from other microbial flora by having ability to balance the osmotic pressure and denaturing effects of high salinity. The halophilic microorganisms use two strategies to withstand high salinity existing in the salterns viz., salt-in and salt-out strategy[3].

Pigments from extremophilic microorganisms, especially halophilic bacteria proved to be the pool of various group of pigments with attractive color and bioactivity. Natural sources are available, microorganisms have proved to be one of the best sources for natural pigments. Halophilic Bacteria isolated from the hypersaline environment produce pigments. Till date, various groups of pigments like carotenoids, prodiginines, quinones, melanins were reported from halophilic bacteria. Pigments protect bacteria from intense sunlight and UV radiation. Most of the halophilic organisms are pigment producers. Some of the pigments belong to prodigiosin, carotenoids and ketocarotenoids.[4].Pigments have lot of medicinal properties in the form of antimicrobial, anticancer, antioxidant and anti-inflammatory activities.[5]

Bacterial pigments are still at the research and development stage. Therefore, it is essential to study and analyze the biodiversity of this solar saltern which would help us to understand the distribution, physiology and biological properties of halophilic bacteria. Hence, the present study focuses on the solar saltern in Marakkanam, Tamil Nadu, India.

MATERIALS AND METHODS

Extraction of Pigmented secondary metabolites

In previous studies, the halophilic bacteria isolated and identified from marakanam saltpan,tamilnadu. The pure culture of all pigment producing halophilic bacteria were grown in 100 mL of halophilic broth was sterilized at 121°C under 15lbs for 20 minutes separately inoculated with selected pigment

producing halophilic bacteria and incubated at 37°C in an orbital shaker under 150 rpm. After three days of incubation, the culture was harvested by centrifugation at 10,000 rpm for 10 min at room temperature. The pigments were extracted using acetone (intracellular) and ethyl acetate (extracellular) besides the type of pigment produced by the isolates. The extracted Pigment were subjected to dry under Vacuum oven for overnight. The dried Pigments were further evaluated for potential analysis.[7]

Antibacterial activity of extracted pigments

Collection of clinical pathogens

Bacterial pathogens such as *Staphylococcus aureus* (ATCC 25923), *Streptococcus pyogens* (ATCC 12384), *streptococcus pneumoniae* (ATCC 25923) *Escherichia coli* (ATCC 25922) *klebsiellapneumoniae* (ATCC 70063) ,*serratiamarcescens* (ATCC-13880) were obtained from ATCC, Bangalore, India. Pathogens were stored in agar slants at 4°C for further use.

Well diffusion method

The antimicrobial activities of Supernatant were evaluated by well diffusion method. Pathogenic bacterial strains (*Staphylococcus aureus, Streptococcus pyogens, streptococcus pneumoniae, Escherichia coli,klebsiellapneumoniae,serratiamarcescens*) were inoculated in Nutrient broth and incubated overnight at 37^0C for exponential growth of culture. Further, the pathogens were swabbed of freshly prepared Mueller-Hinton agar plates. Different volumes viz., 25 µl, 50 µl, 75 µl and 100 µl of dissolved extracted pigments were added to well and the plates were incubated at 37^0C for 24 hours[7].

Characterization of Pigments

FT-IR analysis

The infrared (IR) spectrum of the purified protein was determined by EXI- Spectrum One Model. The spectrum was obtained using potassium bromide (KBr) pellet techniques in the range of 4000 to 400 cm^{-1}at a resolution of 1.0 cm^{-1}.Potassium bromide (AR grade) was dried under vacuum at 100°C and 100 mg of KBr pellet was used. The spectrum was plotted between intensity and wave number. The FT-IR spectra were analyzed for the presence of various functional groups.[8]

GC-MS analysis

GC-MS analysis of crude extract was carried out on Shimadzu 2010 plus comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column RTX 5Ms (Column diameter is 0.32 mm, column length is 30m, column thickness 0.50µm), operating in electron impact mode at 70eV; Helium gas (99%) was used as carrier gas at a constant flow of 1.73 ml /min and an injection volume The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbo Mass Ver 5.2.0. Interpretation on GC-MS was conducted using the database of National Institute Standard and Technology (NIST). The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained. **[9]**

RESULTS AND DISCUSSION

Halophilic bacteria were cultured in halophilic broth and after 7 days, the cells were separated by centrifugation for pigment extraction. The dark yellow color pigmented secondary metabolites from MSP1 and orange color pigmented secondary metabolites from MSP8. saltpan is the highly intensive terms of saline environment and they can harbour truly halophilic bacteria. However, the potential halophilic bacteria present in these environments have several Bioactive compounds from a halophilic bacterium had antimicrobial activity. [10]. Pigments are the source of antioxidants and antibacterial activity, Halorubrum species are highly produced the carotenoid Production and had good antagonistic activity. Halophilic bacteria can be developed as an innovative source of secondary metabolites, isolated from saltpan, Bibi revealed the *Haloduleuniversis* from Saudi to screening the antimicrobial activity by the antagonistic activity method. Bioactive compounds are useful for the control of bacterial diseases [11]. Carotenoids pigment produced by *halorubrumsp* having bioprospecting products. [12].

Table 1: Antibacterial activity of pigmented secondary metabolites

Pathogens	Zone of inhibition (mm)			
	MSP1		MSP8	
	Acetone	Ethyl acetate	Chloroform	Ethyl Acetate
S. aureus	5±1.00	16±0.32	10±1.00	14±1.00
S.pneumoniae	-	16±0.32	-	13±1.00
S. pyogenes	5±1.00	17±0.90	-	15±0.58
E. coli	4±1.00	14±0.58	11±1.00	17±1.53
K. pneumoniae	8±0.58	13±0.55	17±1.53	12±1.00
S. marcescens	10±1.00	12±0.60	18±0.58	18±0.58

-: No zone of inhibition

Figure 1: FT-IR Spectrum of Ethyl acetate Pigmented crude extract

Balouiri was used to determine the antibacterial activity of extracted pigments. The pigmented secondary metabolites produced by halophilic bacteria were tested against the pathogens by well diffusion method on Muller Hinton agar media [13]. The antibacterial activity results of MSP8 ethyl acetate pigmented extract showed maximum zone of inhibition against all the pathogens viz., *streptococcus pyogenes*(17±0.90), *Staphylococcus aureus* (16±0.32), S*treptococcus pneumoniae* (16±0.32), *Escherichia coli* (14±0.58), *Klebsiella pneumoniae*(13±0.55) and S*erratia marcescens* (12±0.60) . The antibacterial activity results of MSP8 ethyl acetate pigmented extract showed maximum zone of inhibition against all the pathogens viz., *serratiamarcescens* (18±0.58), *Escherichia coli* (17±1.53),*streptococcus pyogenes* (15±0.58), *Staphylococcus aureus* (14±1.00), S*treptococcus pneumoniae* (13±1.00) and *Klebsiella pneumoniae*(12±1.00)[Table-1]. Usman carried outorange pigment was subjected to FT-IR done by Kbr and analysed by computerized [14].

FT-IR spectra were taken on FT-IR, Perkin – Elmer instrument in potassium bromide in the range of 4000 – 600 cm^{-1}.[Figure-1].GC-Ms analysis results revealed 1,2,2-Trimethylcyclopropropylamine with retention time 5.992 ,1,2-Propadiene (CAS) Allene with retention time 7.853, trans-beta-ionon-5,6-epoxide 8.560. These three compounds majorly presented in the ethyl acetate pigmented extracts. All the compounds were summarized with their molecular formula, molecular weight, area of peak, retention time. The compounds with more area of peak were further focused in isolation studies. The area of a peak is directly proportional to the amount of the compound that is found in a given mixture. [Figure-2] & [Table-2].

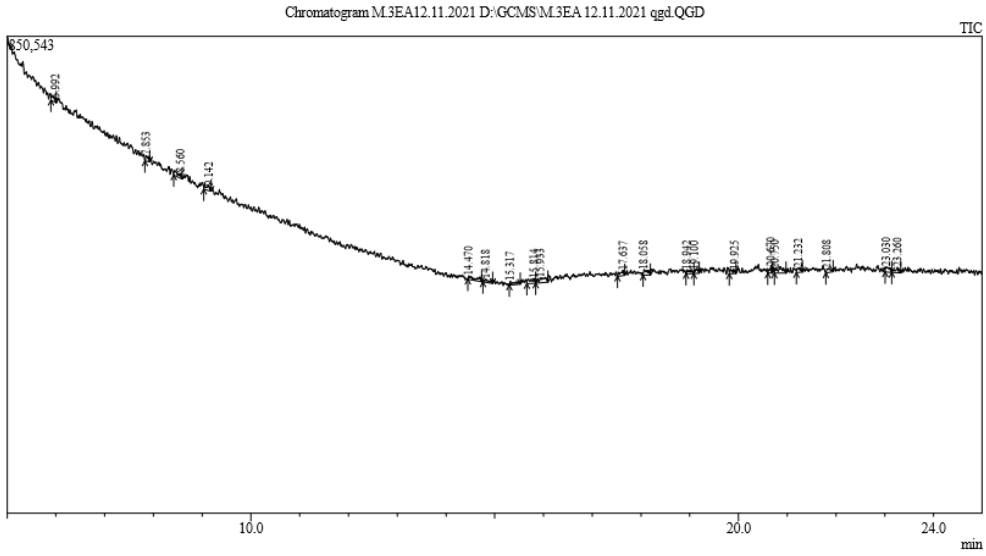

Figure 2: GC-MS Spectrum analysis of MSP8 pigmented ethyl acetate extract

Table 2: Chemical compounds of MSP8 pigmented ethyl acetate extract

S.No	R.Time	Area	Area%	Height	Height%	Name
1	5.992	40205	3.93	7368	4.64	1,2,2-Trimethylcyclopropropylamine
2	7.853	39004	3.81	10040	6.32	1,2-Propadiene (CAS) Allene
3	8.560	62444	6.10	8756	5.51	trans-beta-ionon-5,6-epoxide
4	9.142	46616	4.55	7625	4.80	3S,4S,5S-3-Tert Butoxy carbonyl amino-5
5	14.470	63256	6.18	6511	4.10	5-Propyl-2-Z-4, octadienentrile
6	14.818	35901	3.51	5940	3.74	5,14-[1,2] benzo-5,14,8,11-tetrahydro-8,11
7	15.317	45028	4.40	6995	4.40	5-Methoxyl-1-aza-6-oxabicyclo
8	15.814	62142	6.07	8202	5.16	18-(tert-butyldimethylsilyl)oxyl
9	15.933	93645	9.15	8828	5.56	1,1-bibicyclo (2,2,2)octyl-4-carboxylic acid
10	17.637	46870	4.58	9177	5.78	See- Butyl Phenyl Ketone
11	18.058	60978	5.96	9096	5.72	2,2-dibromo-1-methylcyclopropane
12	18.942	37108	3.63	6563	4.13	7-hydroxy-5,6,7,8-tetrahydroindoliziane
13	19.100	35963	3.51	5830	3.67	N-P-anisidinomethyl
14	19.925	44178	4.32	6310	3.97	Argon
15	20.670	36514	3.57	11233	7.07	Tricarbonyl
16	20.750	90060	8.80	6967	4.38	1,2,5-oxadiazole
17	21.232	38822	3.79	7706	4.85	3,3-Dimethyl-2-phenyl-2
18	21.808	40878	3.99	6394	4.02	Propanoic acid
19	23.030	52999	5.18	9576	6.03	Ethyl-1-Hexyl-4
20	23.260	50933	4.98	9780	6.15	1-propyne
		1023544	100.00	158897	100.00	

CONCLUSION

The study concluded pigment extracted from halophilic bacteria was produce light orange color pigment. Bioactive pigment having great antibacterial activity against all the bacterial pathogens. Further studies including purification, identification of pigment and check other pharmacological studies.

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