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Bacteriostatic Effect On MDR Pathogen, Using Marine Extremophilic *Bacillus Sp.* Via Extremophilic Gene Alteration

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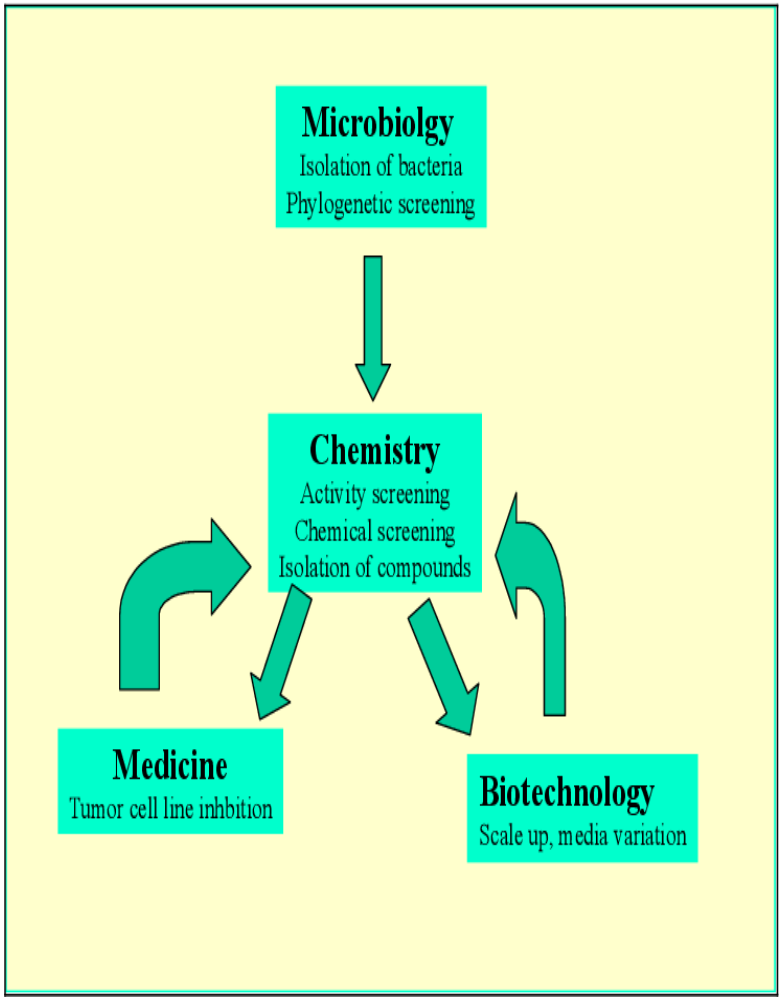
Introduction

- Marine environment sources- biologically active secondary metabolites. Industrial soil/water is rich source of extremophilic and antimicrobial microorganism.
- Marine microbes are able to live in high salt condition. Halophiles are salt tolerant organisms.
- *Escherichia coli*, also known as *E. coli*, is a Gram-negative, facultative anaerobic, rod-shaped, coliform bacterium
- *Staphylococcus aureus*- Gram positive bacterium, found in respiratory tract and skin
- *Pseudomonas aeruginosa* is the pathogenic, facultatively anaerobic Gram-negative bacterium.
- Marine microbes are yet to be investigated for the discovery of novel bioactive compound and novel enzyme



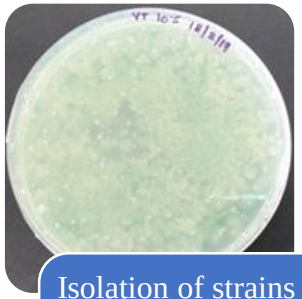
Objectives

- ✓ Isolation and preliminary screening of bacterial strain from marine soil samples
- ✓ Secondary screening and identification of antimicrobial strains.
- ✓ Biochemical and Molecular characterization of antimicrobial isolates by determination of 16s RNA sequence
- ✓ Characterization and purification of antimicrobial compounds from selected bacteria and identification of purified compound using analytical methods
- ✓ Identification of gene alteration of pathogenic bacteria by molecular techniques.

Methodology

Sample Collection

- Collection of soil samples from salt pans

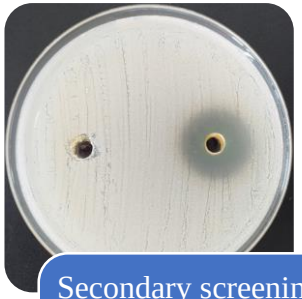


Isolation of strains

- Serial dilution done for selection of potent isolates

Preliminary screening

- potent isolates against clinical pathogens by dual culture assay



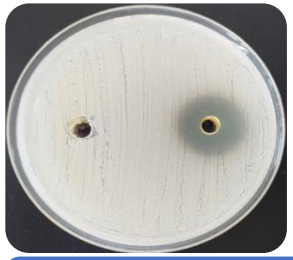
Secondary screening

- using well cut method
- Biochemical characterisation of selected isolate VT-5

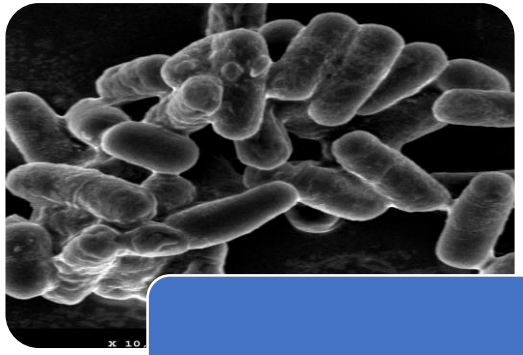
VT-5 was cultured by the shake-flask method,

Extraction of secondary metabolites from isolated strain (VT-5) via liquid-liquid extraction method.

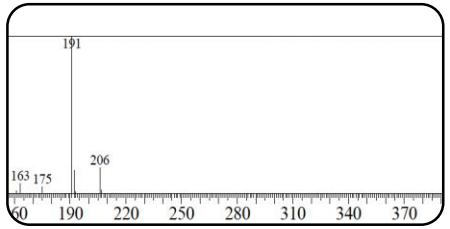
Silica gel Column chromatography of crude extract was performed to collect the all fractions



Antimicrobial assay and spectroscopic analysis was done for fraction-B



FESEM



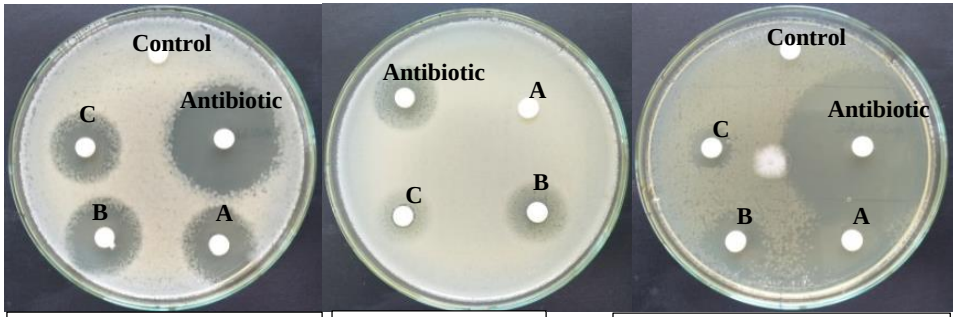
GCMS

Results

Table 1:- Calculation of zone of inhibition for isolated fraction.

Pathogenic Microorganism	Zone of Inhibition (mm)				
	Fraction-A (µl)	Fraction-B (µl)	Fraction-C (µl)	Positive Control(µl)	Negative Control(µl)
Escherichia coli	17mm	19mm	17mm	21mm	NIL
Staphylococcus aureus	NIL	15mm	10mm	15mm	NIL
Pseudomonas aeruginosa	18mm	18mm	8mm	25mm	NIL

Figure 1:- Dual culture assay with disc diffusion method for isolated fractions



Staphylococcus aureus *Escherichia coli* *Pseudomonas aeruginosa*

Staphylococcus aureus *Escherichia coli* *Pseudomonas aeruginosa*

Results

Figure 2 :- 1) TLC for crude Extract. 2) TLC for Fraction-B

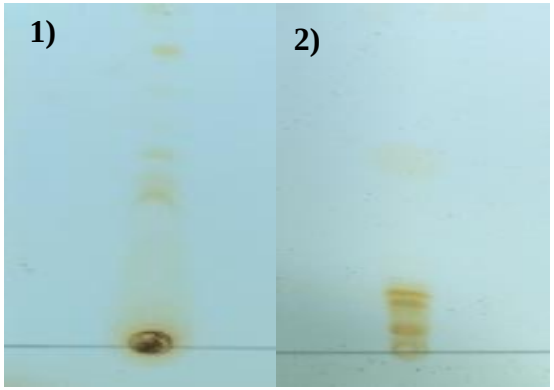
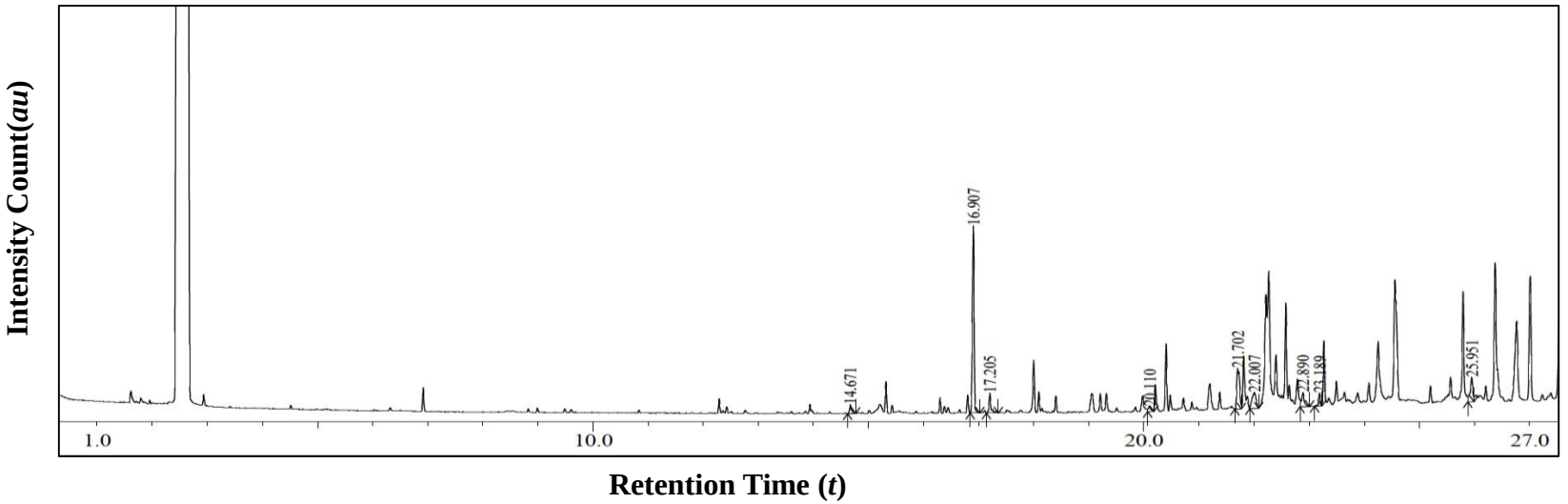


Figure 4:- GCMS- chromatogram for bioactive compounds from *Bacillus halotolrens* (VT-5) of fraction-B



Results

Table 2:- Analysis of fraction-B for bioactive compounds by GCMS

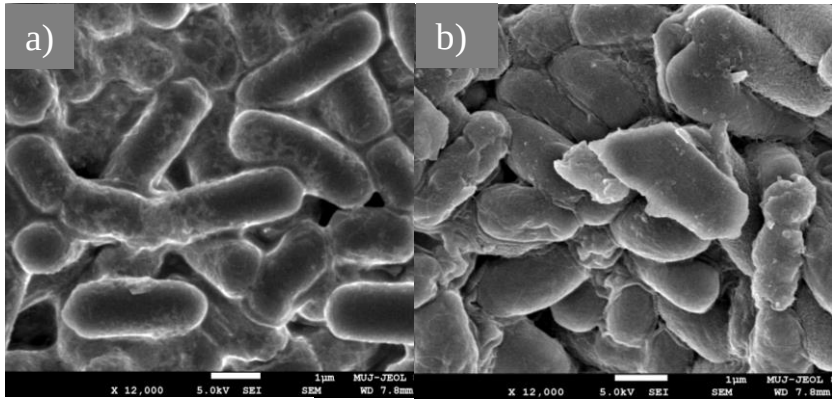
Serial No.	Bacterial bioactive compounds	Retention time	Area %	Molecular weight	Molecular Formula	Biological Properties
1	2,5-Dimethylhexane-2,5-dihydroperoxide	14.671	2.28	178	$C_8H_{18}O_4$	Bioactive compound; Marine, plants and microbes; anti-oxidants, anti-inflammatory and anti-aging compound.
2	2,4-Di-tert-butylphenol	16.907	45.93	206	$C_{14}H_{22}O$	Anti-microbial, anti-oxidant, anti-cancer and marine metabolites
3	2-Butenedioic acid (Z)-, dibutyl ester	17.205	5.76	228	$C_{12}H_{20}O_4$	Phytochemical compound and anti-microbial activity
4	3,5-di-tert-Butyl-4-hydroxybenzaldehyde	20.110	2.03	234	$C_{15}H_{22}O_2$	Anti-microbial compound, anti-proliferative, anti-inflammatory, immunomodulatory, anti-viral, and cytotoxic

Cont..

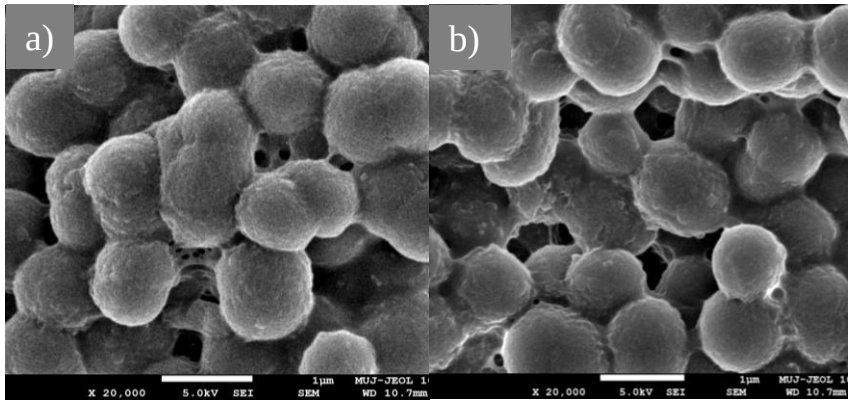
5	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	21.702	17.16	278	$C_{16}H_{22}O_4$	Bioactive compound; plants and microbes; anti-microbial, α-glucosidase inhibition, and in-vivo hypoglycaemic effect.
6	Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	22.007	9.19	210	$C_{11}H_{18}N_2O_2$	Anti-microbial, anti- cancer activity and anti-oxidant potential
7	4,6-di-tert-Butyl-m-cresol	22.890	5.76	220	$C_{15}H_{24}O$	Phytochemical compound; anti-bacterial, anti-fungal, antioxidants, and anti-inflammatory agents
8	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	23.189	3.48	334	$C_{20}H_{30}O_4$	Phytochemical compound, free radical scavenging activity, anti-microbial agent and fungal metabolite.
9	[1,1'-Biphenyl]-2,3'-diol, 3,4',5,6'-tetrakis(1,1-dimethylethyl)-	25.951	8.41	410	$C_{28}H_{42}O_2$	Phytochemical compound; free radical scavenging activity, anti-bacterial potential and anti-cancer agent.

Results

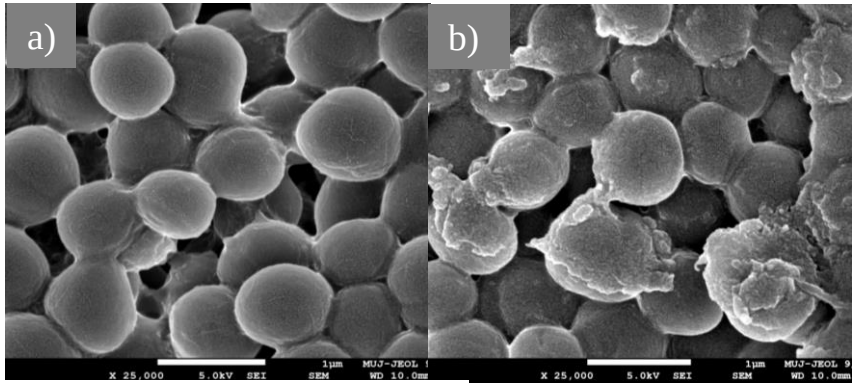
Figure 3: - FESEM of bacterial cell culture ,treated with VT-5 CFS.



Escherichia coli



Pseudomonas aeruginosa



Staphylococcus aureus

a) Before treatment

b) After treatment

Results

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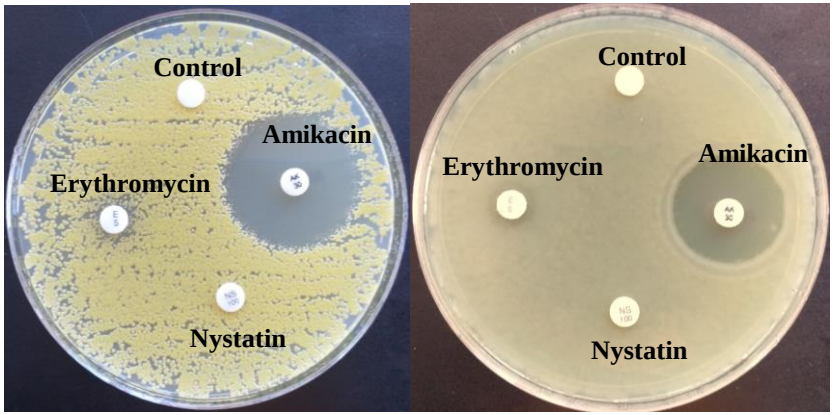
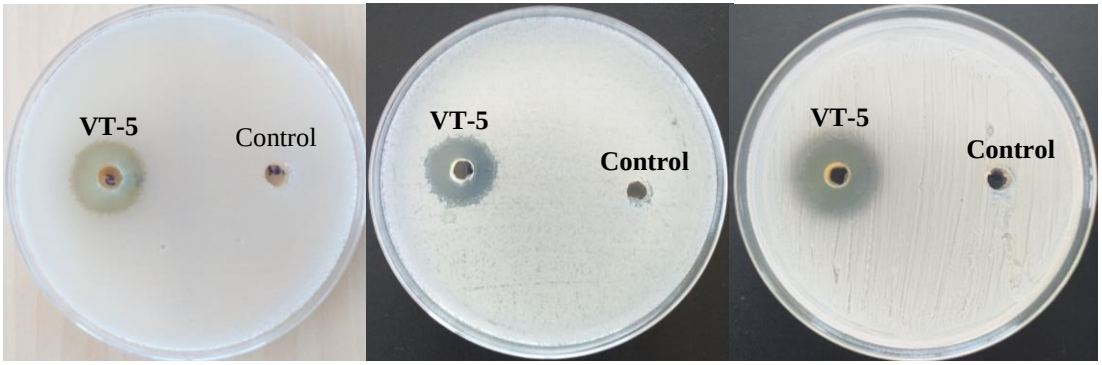
Figure 4 :- MDR (Multi drug resistance) pathogenic bacteria



Pseudomonas aeruginosa ***Escherichia coli***

Figure 5 :- Isolated Strain VT-5 Showing antimicrobial activity against MDR pathogenic bacteria



Escherichia coli ***Staphylococcus aureus*** ***Pseudomonas aeruginosa***

Results

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Table 3 :- Calculation of zone of inhibition for VT-5 against MDR pathogenic bacteria

Pathogenic Microorganisms	**Zone of inhibition (mm)**	
	VT-5 (µl)	Control (µl)
Escherichia coli	17mm	Nil
Staphylococcus aureus	17mm	Nil
Pseudomonas aeruginosa	20mm	Nil

Conclusion

- Total 50 strains isolated from marine sediment Tamil Nadu, India. Strain VT-5 isolated from marine sediments, showed strong antagonistic activity against bacterial pathogens.(*Staphylococcus aureus* , *Escherichia coli, and Pseudomonas aeruginosa*).
- Based on 16S rRNA analysis, the VT-5 strain showed 99.75% homology with *Bacillus halotolerens* (gram +ve, rod shaped, stress, salt and drought resistant bacterium). with accession number ***ON847328***
- Extraction of secondary metabolites was done with 2 solvents; Hexane and Chloroform, where extraction performed with Chloroform showed efficient antibacterial activity. Crude extract was purified by Silica gel column chromatography and antibacterial activity of different fractions was studied.
- 9 major compounds was identified as an active compound with the help of GC/MS.
- Due to the antibacterial efficacy of bioactive compound, the FESEM showed the morphological changes in outer layer of pathogenic microorganisms.
- Our study highlights, 9 major bioactive compounds, as an efficient anti-pathogenic agent. as well as its use in combinatorial therapy to treat persistent and drug resistant infection.

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THANK YOU