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Screening and Identification of Multi-Metal Resistance Halophilic Bacteria from Different Habitats of Odisha, India

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A B S T R A C T

The current study focuses on the screening and identification of halophilic bacteria from different habitats of Odisha for selecting a potentmulti-metal resistance bacterium. The halophilic bacteria were isolated and screened with NaCl concentration using 0%-24% (w/v). The selected halophiles were studied for their growth, colony morphology, sodium content, flavonoid content and multi-metal resistance. The isolated bacteria were found to be moderately halophilic to slight halophilic in nature due to the presence of salt such as sodium, magnesium and bicarbonate in the collected samples. The result showed the utmost number of viable cells (1.65 to 5.40 cfu/mL) is due to the hypersaline nature making the bacterial cells suitable to grow under 15%-18% (w/v) NaCl concentration. They were found to be gram-positive and organized in single rods and clusters. The presence of sodium (0.018-0.249 mg/L) were confirmed with the help of flame photometry in the halophiles. The flavonoid content (0.074-0.330 mg/mL) indicated the presence of antioxidant activity.AS'S-I strain found to be potent multi-metal resistant halophilic bacteria analysed through SEM analysis. Molecular identification of AS'S-I confirmed the *Halomonassp*. The study suggests that AS'S-I halophilic bacteria could be used for the multi-metal removal.

Introduction

Coastal areas are the major site of pollutants due to the increased buildup of refractory elements, notably the accumulation of heavy metals in aquatic environment released by the industries. The major pollutants like heavy metals cause health hazards in the vicinity areas with respect to plants, animals and microbes (Tchounwou *et al.,* 2012; Sahoo *et al.,* 2021; Balali-Mood *et al.,* 2021). Because the sea receives a variety of anthropogenic inputs from various point sources located in near-shore industrial, urban, and metropolitan areas, they serve as the ultimate sink for all pollutants. As a result of all these pollutants, marine sediment pollution and ecological degradation were eventually brought to the surface of the sea sediment through the water column (Caeiro *et al.,* 2005; Dharmendra *et al.,* 2020). The metal flow to the marine environment has increased recently, and this had a significant impact on the coastal ecosystems due to the industrial and urban activities (Ferati *et al.,* 2015; Harikrishnan *et al.,* 2017; Swain *et al.,* 2021; Diba *et al.,* 2021). The bioremediation of

metals from high-salinity waste water and industrial effluents, particularly when NaCl is present has received little research attention.

Under these circumstances, marine microorganisms that can withstand salt are more advantageous than traditional microorganisms because they can quickly adapt to a changing environment.

This saline environment is the ideal habitat for bioremediation since these marine microorganisms has diverse metabolisms (Shukla *et al.,* 2017; Mohapatra *et al.,* 2017**;** Matarredona *et al.,* 2021; Diba *et al.,* 2021). Bacterial cell walls comprise a variety of surface organic functional groups, which offer high affinity to binding of metals.

Hence, biosorption of heavy metals by bacterial cell wall has been in practise for a long time (Daughney *et al.,* 2002; Feng *et al.,* 2012**;** Saranya *et al.,* 2018). They have the ability to grow under extreme saline conditions having potential mitigation properties against hazardous pollutants.

The halophilic bacteria use 'compatible solutes adaptation strategy' to maintain osmotic balance by using compatible organic solutes such as polyols, glucosylglycerol, sucrose, trehalose, ectoine, and betaine. Halophilic bacteria have unique metabolic capabilities, under extreme hostile conditions, and unique biomaterials and/or secondary metabolites (Shukla *et al.,* 2017; Weinisch *et al.,* 2018; Leon *et al.,* 2018; Corral *et al.,* 2019; Van Thuoc *et al.,* 2021; Matarredona *et al.,* 2021; Baati *et al.,* 2022). Therefore, this study explores the different habitats of Odisha (Astaranga, Puri and Baulabandha, Chilika) for screening of potent halophilic bacteria having the potential of metal removal. So, for that isolated bacteria were screened for halophilic nature and multi-metal resistance through NaCl and different metal concentrations. Then, they are qualitatively and molecularly characterized for identifying the suitable halophilic bacteria.

Materials and Methods

Physico-Chemical Characterization

Water and soil samples were collected from different coastal areas of Odisha. Astaranga (19.929°, 86.283°) and Baulabandha, Chilika (Lat 19.801°, Long 85.325°) of Odisha. Temperature, pH, TDS, redox potential and electrical conductivity of the water samples were taken immediately with the help of digital meter (Vahed *et al.,* 2011**;** Chun-Ming *et al.,* 2011; Lu *et al.,* 2015; Das *et al.,* 2019**;** Diba *et al.,* 2021) and for soil samples these parameters were measured as per the method described by Selvarajan *et al.,* (2017) and Sharma *et al.,* (2021) which were stored in sterile plastic bottles at 23℃.

Thereafter, cation and anion analysis were performed. Sodium, potassium and calcium were measured as per the Gharaibeh *et al.,* (2021) and El Bilali *et al.,* (2021) with the help of flame photometry. Magnesium, chloride, carbonate, bicarbonate and dissolved oxygen was measured according to the Gharaibeh *et al.,* (2021) and El Bilali *et al.,* (2021) using titration method. Sulphate and nitrate were measured with spectrophotometric method of Mussa *et al.,* (2009) and Dookie *et al.,* (2022). All the Physico-chemical characteristics were performed in triplicates.

Isolation and Screening of Halophilic Bacteria

The halophilic medium C was used for halophilic bacterial isolation in agar plates under incubation of 35℃ (Rohban *et al.,* 2009) for 24 to 48 hours. Under medium C (per liter): NaCl, 81g; $MgSO_4.7H_2O$, 9.7g; $MgCl_2.6H_2O$, 7g; $CaCl_2.2H_2O$, 3.6g; KCl, 2g; $NaHCO_3$, 0.06g; $(NH_4)_2SO_4$, 0.026g; Yeast extract, 50g; Agar, 12-15g), the bacteria were isolated in triplicates.

The pure culture of bacterial colonies growing on solid media were determined based on the shape, surface, elevation, colour and margin. The selected strains were screened under NaCl (0% to 24% (w/v)), to know the nature of halophiles (slightly, moderately or extremely) (Das *et al.,* 2019; Sharma *et al.,* 2021; Rathakrishnan and Gopalan, 2022). Cell morphology was observed under phase contrast microscope (model- Nikon) at 100X using the gram stain method (Fitri *et al.,* 2022).

Qualitative Characterization of Halophilic Bacteria

Sodium Capture Capacity

The positive isolates were selected for sodium uptake capacity and pigment characterization. Potential isolates growing luxuriantly under 19% and 17% NaCl were screened for sodium uptake content. After 24 hours, cells were harvested by centrifugation (Optima MAX-XP, Bekman, USA) (12,000 rpm) and the bacterial cell pellet

was washed with sterilized distilled water to remove the traces of medium. Washed pellet was digested overnight (0.1 N HCl) at room temperature and again, centrifuged. The supernatant was taken for the estimation of sodium uptake by bacterial cells. Sodium contents were measured by Flame photometer as per the methodology given by the Damodaran *et al.,* (2013) and Pérez-Inocencio *et al.,* (2022). This analysis was performed in triplicate.

Total Flavonoid Content

For flavonoid determination, the cell free supernatant was obtained on separation of the cells by centrifuging at 12000×g for 20 minutes at 4℃ for three times in ethyl acetate, concentrated to dryness under vacuum condition. Individual crude extracts were weighed and dissolved in methanol to get a 1000 µg/ml test solution of crude extracts. All the experiments were carried out in triplicate and appropriate solvent control was maintained (Velho-Pereira *et al.,* 2015). The total flavonoids in the extract were measured using a 96 well microplate reader (CYTATION5 imaging reader, BioTek). Briefly, 50 mL of each extract was mixed with 200 mL of 10% aluminium chloride and 1 M of sodium acetate and incubated for 20 minutes. The absorbance was measured at 450 nm where quercetin (200 to 1000 mg/mL) was served as the positive control. Quercetin (10 to 50 mg/mL) was used as the standard. The scavenging activity (in percentage) of the standard quercetin and extracts were calculated using the following formula: -

Radical scavenging activity (QE) = (Qc -Qs /(Qc) x 100

where Qc and Qs are the absorbance of the control and sample (methanol and extracts), respectively. The quercetin equivalence (QE) is defined as the amount of metabolite present in a specified concentration of the extract that exhibit equivalent activity to that of quercetin (as derived from the slope of the quercetin standard curve) and is expressed in mg/mL (Prathiba and Jayaraman, 2018; Subramanian and Gurunathan, 2020).

Screening for Multi-metal resistance Halophilic Bacteria

Bacterial strains were screened for tolerance under heavy metals which includes Cd, Cr, Mn, Fe, Ni, As, Co, Cu, Zn and Pb. The heavy metal sources are As_2O_3, $Cr_2(SO_4)_3$, $CoSO_4.7H_2O$, $3CdSO_4.8H_2O$, $CuSO_4.5H_2O$, $Fe_2SO_4.7H_2O$, $ZnSO_4.7H_2O$, $MnSO_4.H_2O$, Pb $(NO_3)_2$ and $NiSO_4.6H_2O$. Bacterial strains were seeded on agar plates for screening under the multi-metal (0 to 150 ppm) for incubation period of 24 hours at 35℃.

The bacterial strains were amended with 10 mg L^{-1} to 150 mg L^{-1} (v/v) of multi-metals and incubated at 35°C for 24 hours at 100 rpm. The growth pattern was calculated by measuring the OD at 600 nm (Mathivanan and Rajaram, 2014; Biswas *et al.,* 2017; Sodhi *et al.,* 2020**;** Krishnamurthy *et al.,* 2020; Halder *et al.,* 2022).

FESEM Analysis

Bacterial cell pellets collected from centrifugation at 10,000 rpm for 10 minutes were fixed with 1% (v/v) of glutaraldehyde and 2% (v/v) of paraformaldehyde buffered with 0.1 M of sodium phosphate buffer (pH 6.8) for 4-6 hour at 4°C followed by washing with fresh buffer. Post fixation with osmium tetroxide (1%) in the same buffer at 4°C for 2 hours. Then, again washed with phosphate buffer saline (pH 6.8) followed with dehydration with 30%, 50%, 70% and 90% ethanol for 5 minutes each. Then, dried for 20 minutes under a CO_2 atmosphere. Bacterial cells were mounted with aluminium stubs and were coated with 90-Å-thick gold palladium (VG Microtech, East Sussex, TN22, England) for 30 minutes. Their morphology was observed using field emission scanning electron microscopy (Model-Zeiss EVO40) at 5 kV for studying the biosorption of multi-metals by selected strain (Nithya *et al.,* 2011; Mishra *et al.,* 2011**;** Ma *et al.,* 2020**;** Sahoo *et al.,* 2021).

Molecular Identification

The isolation of genomic DNA and sequencing of 16S rRNA was from Eurofins Genomics India Pvt. Ltd, Bengaluru. The genomic DNA was extracted using commercially available QIAamp DNA Mini Kit (Qiagen).

The quality and quantity of the extracted DNA samples were checked on nanodrop followed by agarose gel electrophoresis. The extracted DNA samples were identified on the basis of molecular identification by targeting bacterial 16S region using Sanger sequencing technique. The sequence obtained were analysed and identified using BLAST search and were compared against bacterial 16S rRNA sequence available on NCBI database.

Data analysis

The data were statistically analyzed using MS excel 2007. Data were subjected to mean ± SD in all parameters using three replicates. Heat map was prepared using conditional formatting inMS excel 2007.

Results and Discussion

Physico-Chemical Characterization

Astaranga samples were found to be saline with EC value of 3.19 to 5.26 ds/m and pH of 5.5 to 7.1. Other physico-chemical parameters such as temperature (˚C), redox potential (mv), TDS (ppm) and DO (Mg/L) was found to be as 32.3 to 32.5, 47.5 to 0073, 3073 to 5828 and 0.22 to 1.25, respectively. Baulabandha samples was found to be slight saline having EC value of 2.94to 3.05ds/m and pH of 8.95 to 2.84. Other physico-chemical parameters such as temperature, redox potential was found to be 33 to 34.5 ˚C, 164 to 233.5 mv, respectively while TDS and DO was found to be as 5564 to 1884ppmand 1.35 to 1.52Mg/L, respectively (Table 1). Magnesium concentration was highest in Astaranga (20380 to 35006.67 Mg/L) and Baulabandha samples (3308.06 to 32210 Mg/L) while calcium content was found to be lowest in Astaranga (0.25 to 49.751 Mg/L) samples and Baulabandha samples (0.35 to 33Mg/L), respectively. Similarly, nitrate was found to be lowest in Astaranga (0.22 Mg/L) and Baulabandha samples (0.17 to 0.20 Mg/L), respectively. Bicarbonate content was highest in Astaranga soil sample (385830 ± 531941.6 Mg/L) and Baulabandha soil sample (206913.3 ± 56093.17 Mg/L) while chloride concentration was highest in Astaranga water sample (57977.4 ± 270.19 Mg/L) and Baulabandha water sample (15015 ± 82.17 Mg/L), correspondingly (Table 2).

Isolation and Screening of Halophilic Bacteria

The highest CFU was recorded in Astaranga soil sample while the lowest was recorded in Baulabandha soil sample, correspondingly (Table 3). A total of 11 bacterial strains were isolated from water and soil samples. As per the morphological study, 8 strains isolated from Astranga samples (AS-I, AS-II, AS-III, AS-IV, AS-V, AS'S-I, AS'S-II, AS'S-III) and 6 strains isolated from Baulabandha samples (BB-I, BB-II, BB'S-I, BB'S-II, BB'S-III, BB'S-IV). All the strains showed NaCl tolerance above 15% and 5 (AS-I, AS'S-I, BB-I, BB'S-III) strains showed NaCl tolerance up to 19% making them moderately halophilic in nature (Table 4). The selected 8 strains (AS-I, AS-II, AS-III, AS'S-I, BB-I, BB-II, BB'S-II, BB'S-III) were studied for their maximum cell growth. The maximum reach of growth was up to 18% NaCl concentration (AS-I, AS'S-I, BB'S-III) which was declined with increasing concentration (figure 1) observed through the heat map. When these examined under a phase contrast microscope, they found to be rod-shape and gram-positive bacteria (figure 2).

Qualitative Characterization of Halophilic Bacteria

Sodium Capture Capacity

On the basis of the growth study, AS-I, AS'S-I, BB'S-III was further studied for characterization. Analysis of these isolates for sodium uptake capacity at 21% NaCl concentration showed higher uptake of sodium content in comparison to control treated Bacteria (figure 3). However, lower uptake of sodium by AS'S-I, BB'S-III strains (0.159 mg/L, 0.098 mg/L) was seen than AS-I (0.249 mg/L), respectively which showed higher uptake of sodium.

Total Flavonoid Content

The total flavonoid content of the selected isolates was found in the range of 0.297 to 0.061 mg/mL (Figure 4). All methanolic extracts of the strains showed highest flavonoid content in control condition than the treated condition. AS'S-I extract showed the highest flavonoid content than other extracts with 21% NaCl treated (0.146 mg/mL) as well as in control treatment (0.330 mg/mL). The presence of flavonoid content in the extract could have contributed to the antioxidant activity. While BB'S-III strain showed lowest flavonoid content among both the condition of the 21% NaCl (0.074 mg/mL) treatment as well as in control treatment (0.135 mg/mL), respectively.

Screening for Multi-metal Resistance Halophilic Bacteria

At different multi-metal concentration, the bacterial cell growth was observed for 24 hours. The bacterial cell growth was seeming to decline with increasing concentration of multi-metal (Figure 5).

Table.1 Physico-chemical characterization

Sample	pH	Temperature (°C)	EC (ds/m)	Redox Potential (mv)	TDS (ppm)	DO (Mg/L)
As water	7.1 ± 0.14	32.55 ± 0.07	5.26 ± 0.20	0073 ± 1.41	5828 ± 5415.02	1.25 ± 0.02
As soil	5.5 ± 0.70	32.3 ± 1.83	3.19 ± 0.09	47.5 ± 0.70	3073 ± 8.48	0.22 ± 0.01
BB water	8.95 ± 0.21	33 ± 1.41	3.05± 0.07	146 ± 2.82	5564 ± 494.97	1.35 ± 0.21
BB soil	2.84 ± 0.06	34.5 ± 0.70	2.94± 0.07	233.5 ± 4.94	1884 ± 16.97	1.52 ± 0.04

As- Astaranga, BB-Baulabandha

Table.2 Cation and Anion analysis

Sample	Na	K	Ca	Mg	Cl	SO_4^{2-}	NO_3	HCO_3^-	CO_3^{2-}
As water	14321.67 ± 10776.92	234.9 ± 15.8	49.751 ± 0	20380 ± 4093.55	57977.4 ± 270.19	4.12 ± 0.02	0.22 ± 0.00	22020 ± 17318.23	206.66 ± 105.03
As soil	9.067 ± 4.45	5.4 ± 0.9	0.25 ± 0	35006.67 ± 10744.49	2106 ± 0	4.20 ± 0.06	0.22 ± 0.00	385830 ± 531941.6	526.66 ± 215.01
BB water	3.151 ± 0	64.55 ± 0.25	33 ± 20	3308.06 ± 2459.40	15015 ± 82.17	4.22 ± 0.05	0.17 ± 0.00	8063.33 ± 144.33	206.66 ± 92.37
BB soil	395 ± 407.11	24.2 ± 15.6	0.35 ± 0	32210 ± 11596.09	7121.4 ± 3377.49	4.19 ± 0.06	0.20 ± 0.00	206913.3 ± 56093.17	3706.66 ± 184.75

As- Astaranga, BB-Baulabandha

Table.3 Isolation of Bacteria

Sample	Dilution factor	No. of colonies	Colony forming unit (CFU/mL)
As water	10^0	33	1.65
As soil	10^0	108	5.40
BB water	10^0	35	1.75
BB soil	10^{-2}	44	0.022

As- Astaranga, BB- Baulabandha

Table.4 Screening of halophilic bacteria under NaCl concentration (0% to 24%)

Bacterial strains	NaCl Concentration (%)						
	0%	3%	12%	15%	18%	21%	24%
AS-I	-	+++	+++	+++	+	+	-
AS-II	-	+++	+++	+++	+	-	-
AS-III	-	+++	+++	+++		-	-
AS-IV	-	+++	+++	+	-	-	-
AS-V	-	+++	++	+	-	-	-
AS'S-I	-	+++	+++	+++	+	-	-
AS'S-II	-	+++	+++	+++	+	-	-
AS'S-III	-	+++	+++	+	-	-	-
BB-I	-	+++	+++	+++	++	+	-
BB-II	-	+++	+++	+++	++	+	-
BB'S-I	-	+++	+++	+++	+	-	-
BB'S-II	-	+++	+++	+++	++	+	-
BB'S-III	-	+++	+++	+++	++	+	-
BB'S-IV	-	+++	+++	++	+	-	-

High growth (+++), low growth (+), no growth (-)

Figure.1 Bacterial cell growth under NaCl concentration (0% to 24%)

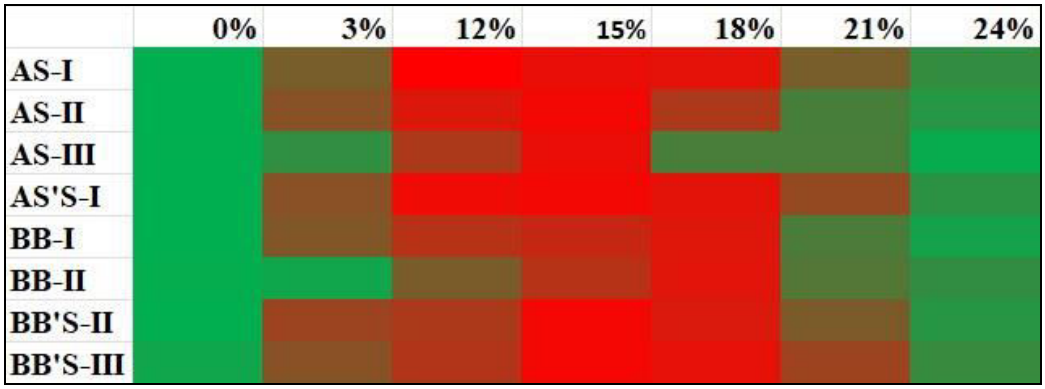


Figure.2 Colony morphology of halophiles

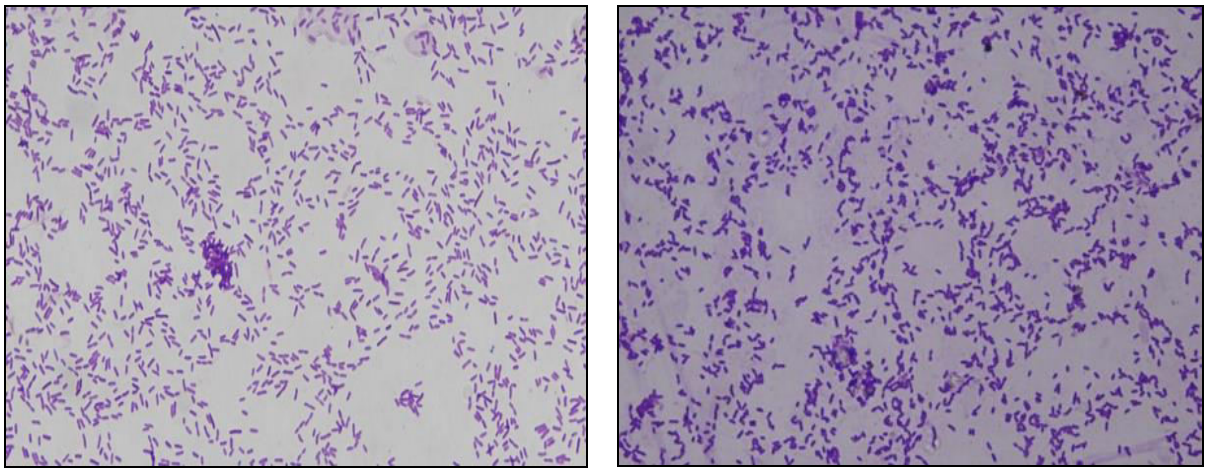


Figure.3 Sodium capture capacity of the halophiles using flame photometry.

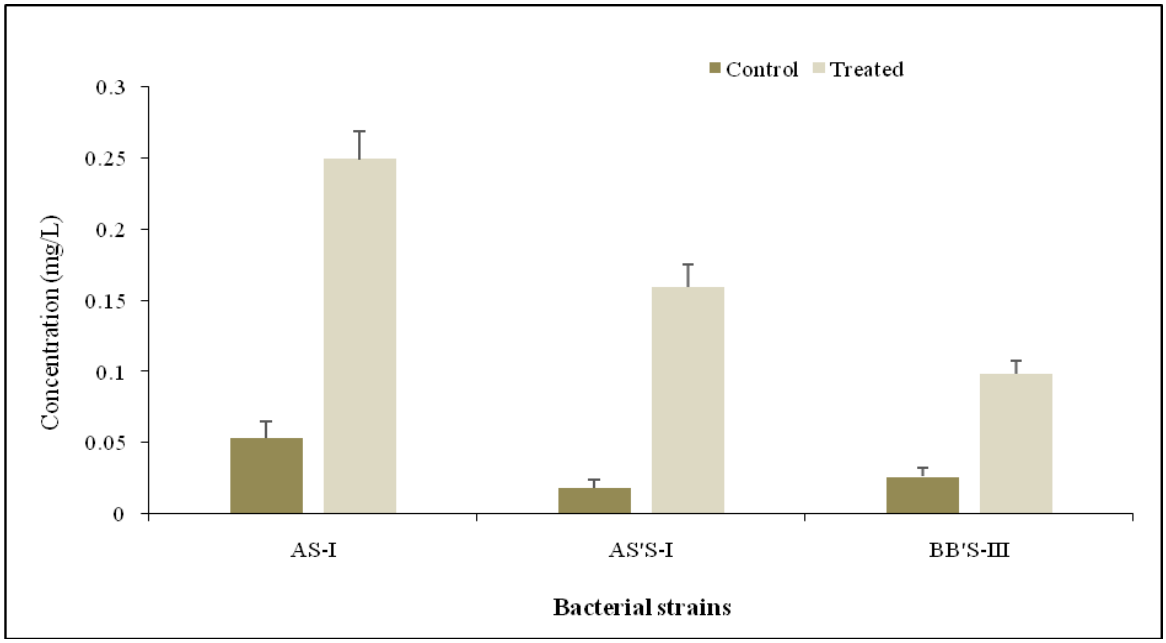


Figure.4 Flavonoid content of halophiles under control and 21% NaCl treatment.

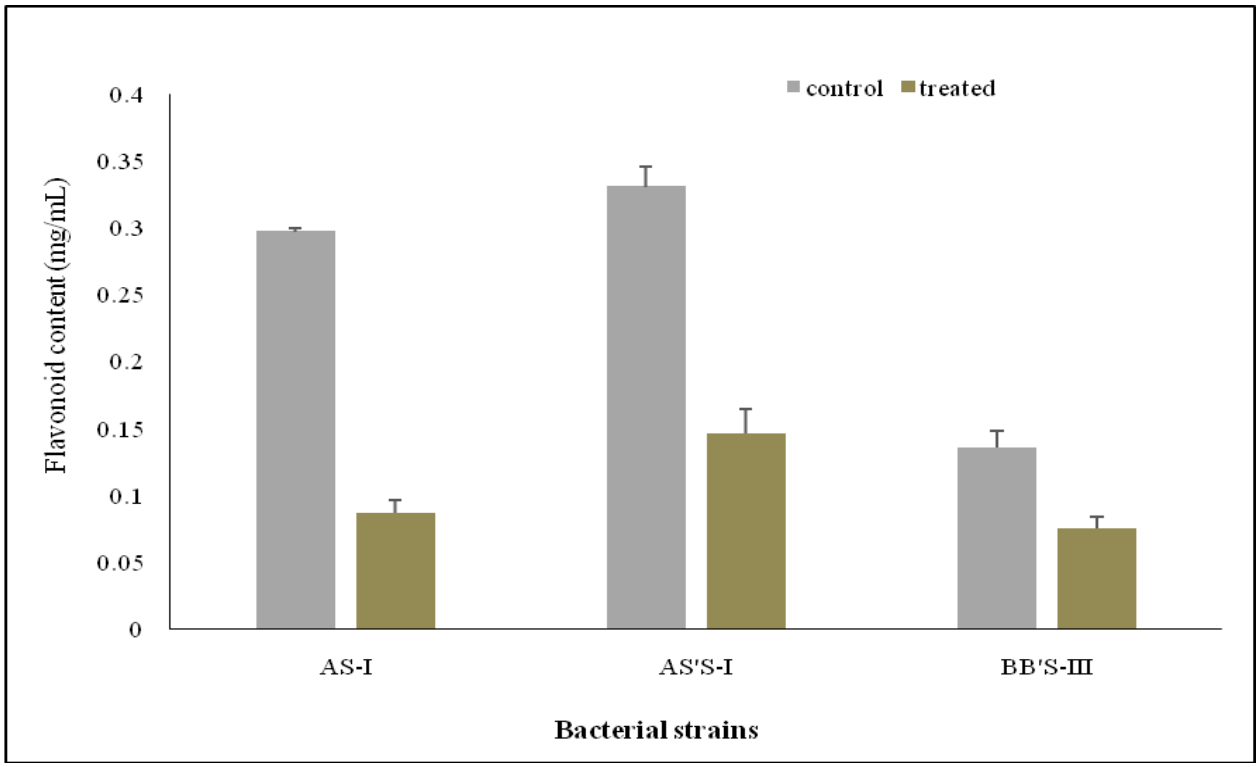


Figure.5 The bacterial cell growth under multi-metal concentration (10-150 ppm (v/v))

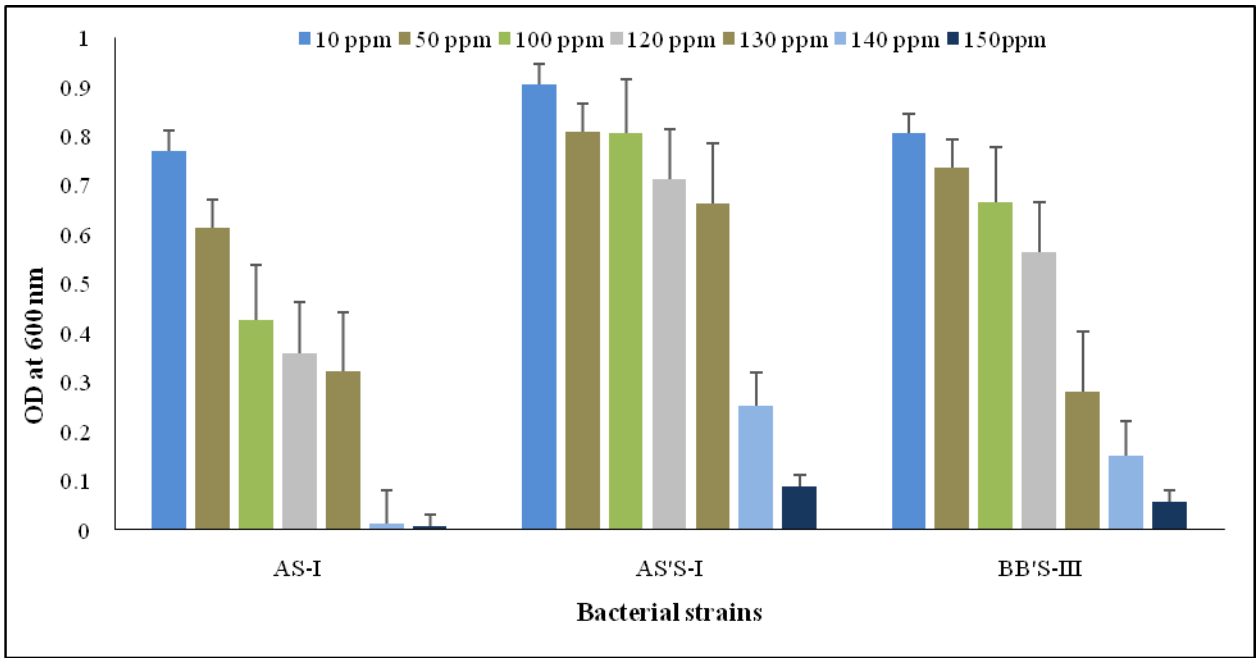
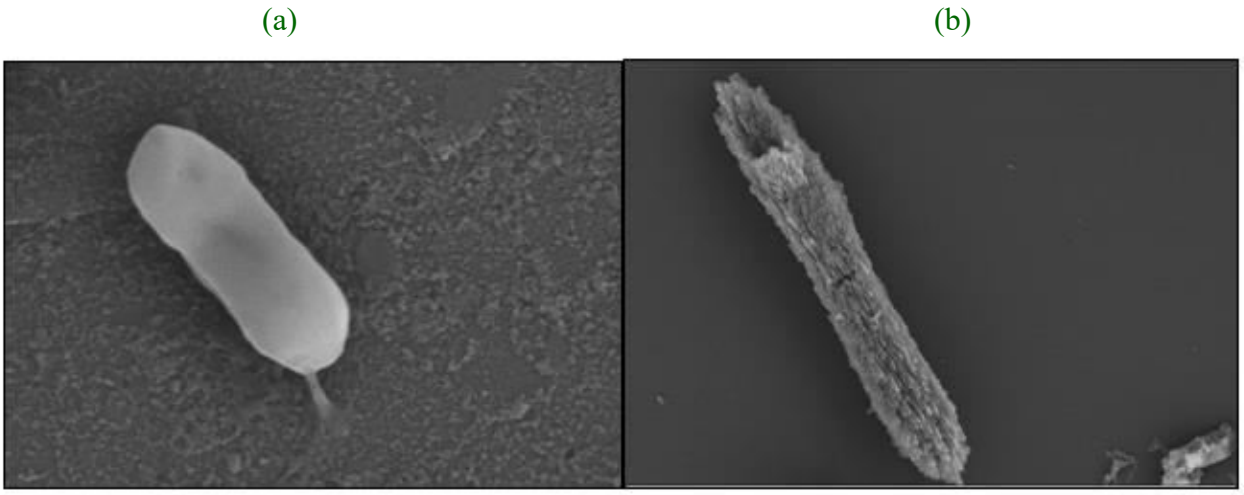


Figure.6 Scanning electron microscope (SEM) micrograph of bacterial strain (AS'S-I) before (a) and after (b) multi-metal absorption.



The bacterial cell found to resist up to 130 ppm multi-metal concentration. There was decline in the growth of AS-I (0.008 to 0.77 Au) as compared to AS'S-I (0.089 to 0.905 Au) and BB'S-III (0.056 to 0.805 Au). AS'S-I seems to grow luxuriantly between 10 ppm (0.905 Au) to 130 ppm (0.663 Au) thereby decline in the growth. After, AS'S-I growth, BB'S-III grows profusely up to 120 ppm (0.805 to 0.563 Au) multi-metal concentration. Decreased growth was observed in AS-I strain.

Biosorption of Multi-metals by Halophilic Bacteria

The potent halophilic bacteria treated with synthetically prepared multi-metal solution (130 ppm) was observe under scanning electron microscopy to analyse the biosorption of multi-metal to the cell surface (Figure 6). There was visible evidence of binding of metal ions unto the surface of the bacteria. Binding of metal ions to the cell surface showed the efficacy of metal resistance of the bacteria.

Molecular Identification

The highly potent multi-metal resistance strain AS'S-I was subjected to 16S rRNA gene sequence analysis. The 16S rRNA gene of the selected isolate was successfully amplified using PCR, and approximately 2 Gb of the amplified products were sequenced. The BLAST-N comparison of the searched sequences in the NCBI nucleotide database revealed 99% similarity of the isolate AS'S-I with *Halomonas sp*. (NCBI accession number: MK129413.1).

The present work focuses on the screening and identification of halophilic bacteria from different habitats of Odisha for selecting a potent multi-metal resistance bacterium. Our present work explores different habitats of Odisha (Astaranga, Puri and Baulabandha, Chilika) for screening of potent halophilic bacteria having the potential of multi-metal resistance.

Physicochemical analysis showed Astaranga samples are saline in nature which indicates the presence of maximum salt concentration in comparison to other samples. Baulabandha samples showed slight saline nature which indicates freshwater can be saline if they are in close vicinity of coastal areas. The salinity of the water at Puri, Digha and Haldia was tested and found to be 34 ppt, 35ppt and 33 ppt, respectively (Das *et al.,* 2019). The Cuatro Cienegas soils had a neutral pH, EC of 2.3e8 dS cm^{-1}, classified as moderately saline. Whereas, the soils from Sayula and San Marcos lakes, had an alkaline pH, EC 15 to 65 dS m^{-1} classified as saline-sodic soil (Delgado-García *et al.,* 2018). A total of 11 bacterial strains were isolated from water and soil samples. All the strains showed NaCl tolerance above 15% and 5 (AS-I, AS'S-I, BB-I, BB'S-III) strains showed NaCl tolerance up to 19% NaCl concentration making them moderately halophilic in nature. The maximum reach of growth was up to 18% NaCl concentration for AS-I, AS'S-I and BB'S-III. These strains are found to be rod shaped and gram-positive bacteria. Diba *et al.,* (2021) reported that

the isolated halophilic bacteria (*Bacillus* sp. A21, *Oceanobacillus* sp. A22 and *Salinicoccus* A43) from Khara Salt Lake in Iran resisted to 20% salt concentration. Similarly, Das *et al.,* (2019) has reported 7 isolates endured 8-20% salt indicating them as extremophiles isolated from the locations of West Bengal and Odisha. A pigment producing moderately halophilic bacterial strain *Halobacillus trueperi* MXM-16 was isolated from mangrove plant litter of Goa (Kharangate-Lad and Bhosle, 2016). Moderately halophilic *Vigri bacillus* sp. isolated from mangrove soil of Bhitarkanika, India showed high salt (25 wt.% NaCl) and Cr(VI) (1000 mg L−1) tolerance. Harmesh Sahay *et al.,* (2011) isolated 51 strains, tolerant to 5% or more NaCl which were grouped into 29 clusters. They are *Bacillus*, *Virgi bacillus*, *Rummeli bacillus*, *Alkali bacillus*, *Halo bacillus, Salimicrobium halophilum*, *Halomonas salina*, *H. shengliensis*, *H. salifodinae*, *H. pacifica*, *H. aquamarina* and *H. halophila* belonging to Firmicutes and γ-Proteobacteria. Chen *et al.,* (2010) reported 45 moderately halophilic (5 to 15% salinity) bacteria isolated from sediment and saline water collected from the Weihai Solar Saltern, China. The isolated strains were related to the phylum Firmicutes and belonged to four genera, *Bacillus*, *Halo bacillus*, *Planococcus* and *Salinicoccus*. The other strains identified as genus of *Halomonas* belonged to phylum γ-Proteobacteria.

The selected strains are further studied for their qualitative properties like sodium capture capacity and flavonoid content. The presence of sodium in bacterial cell helps them to maintain the osmotic balance. Damodaran *et al.,* (2013) reported that analysis of the sodium uptake pattern at different molar concentration of NaCl showed an increasing sodium uptake up to 1 M NaCl in all the isolates thereby significant decline in sodium content. Among them, *Bacillus pumilus* and *Bacillus subtilis* showed higher uptake of sodium (1.272 meq/L and 1.122 meq/L) at 1 M NaCl concentration, respectively. Pérez-Inocencio *et al.,* (2022) reported that the isolates that showed sodium uptake content were *Bacillus* sp. SVCN1, *Bacillus* sp. SVHM1.1, *Bacillus* sp. SVHM9, *Bacillus subtilis* SVCN10, *Bacillus subtilis* SVHM10, *Oceanobacillus* sp. SVCN2, *Oceanobacillus* sp. SVHM7, *Staphylococcus epidermidis* SVHM1, *Marinococcus* sp. SVHM5, *Nocardiopsis* sp. SVHM6.2, *Halomonas* sp. SVCN3, *Halomonas* sp. SVCN8, *Halomonas* sp. SVHM3, *Halomonas* sp. SVHM8, *Halomonas huangheensis* SVCN7 and *Halomonas huangheensis* SVHM6, ranging from 11 mEq (*Bacillus subtilis* SVHM10) to 38 mEq with higher production (*Halomonas huangheensis* SVHM6) after 24 h of incubation.

Multi metal resistance experiment was carried out on the strains AS-I, AS'S-I, BB'S-III. The AS'S-I, BB'S-III strains were found to resist up to 130 ppm multi-metal concentration. Among them, AS'S-I strain was found to exhibit good growth in media amended with 130 ppm multi-metal (As, Fe, Pb, Ni, Mn, Co, Cd, Cr, Cu and Zn) concentration. Orji *et al.,* (2021) revealed, the bacterial isolates showed capacity to resist 50.0 mM Hg and Pb, 17.0, 12.50 mM and 4.0 mM Ni, Cd, and Zn, respectively in solid media. *Pseudomonas putida* A4W Strain also resisted 16.0 mM Cu, while *Klebsiella* sp. Strain USL2S, *Pseudomonas putida* USL5W Strain resisted 4.0 mM each. Sahoo and Goli (2020) stated that *Bacillus pumilus* out of 128 screened bacteria for resistance against Pb, Cd, Ba, Cr, Fe and Cu was found to be resistant against all of them. Abbas *et al.,* (2016) reported that *Proteus* sp. NA6 was found to resist different metal ions (Co, Cr, Zn, Pb, Cu and Cd), respectively.

The AS'S-I strain was observed under the scanning electron microscopy to analyse the biosorption of multi-metals to the cell surface. There was clear visibility of binding of metal ions unto the surface of the bacteria which presents the efficacy of metal resistance. Similarly, Sodhi *et al.,* (2020) showed the absorption of Cu, Cd, Cr, Ni and Zn through SEM-EDX analysis in *Alcaligenes* sp. MMA. Three species of *Bacillus* isolated from solar salterns (*Bacillus licheniformis* NSPA5, *Bacillus cereus* NSPA8, and *Bacillus subtilis* NSPA13) showed significant level of lead biosorption with maximum of 87–90% by *Bacillus cereus* NSPA8. The biosorption of Cu and Cr was relatively low in comparison to lead given by Syed and Chinthala (2015).

The BLAST-N comparison of the searched sequences in the NCBI nucleotide database revealed 99% similarity of the isolate AS'S-I with *Halomonassp*. Yin *et al.,* (2022) reported that *Halomonas salinarum* sp., a moderately halophilic bacterium isolated from saline soil in Yingkou, China grew in the presence of 3–15% (w/v) NaCl concentration. Several *Halomonas* species reported by SarÂyar-Akbulut *et al.,* (2008); Rohban *et al.,* (2009) and Sumit Kumar *et al.,* (2012) has been identified as halophilic bacteria.

The outcomes of the current findings suggests that AS'S-I is a moderately halophilic bacteria having sodium capture capacity and adequate flavonoid content as anti-

oxidant property. AS'S-I presented resistance up to 130 ppm multi-metal concentration and molecular identification revealed the strain as *Halomonas sp*. The metal biosorption competence was further established with SEM coupled with EDS to ascertain surface adsorption of the metal onto the bacterial cell surface. It is evident that the halophilic bacterium AS'S-I is a potential heavy metal removal strain. This can be used for metal removal from effluents of industries present in near shore areas as well as removal from agricultural land which in turn would enhance the growth of crops. Further investigation is needed to understand the potential mechanism of heavy metal detoxification.

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Author Contributions

Itishree Behera: Investigation, formal analysis, writing—original draft. Umesh Chandra Naik: Validation, methodology, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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