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FORTIFICATION OF LAUNDRY WATER WITH BACTERIA CAPABLE OF SODIUM DODECYL SULFATE (SDS) REMEDIATION AND PLANT GROWTH PROMOTION. A SUSTAINABLE WAY TO REUSE WATER FOR IRRIGATION

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ABSTRACT

Anionic surfactant sodium dodecyl sulfate (SDS) is used in cosmetics and cleaning goods. It discharges into the environment and waterways due to its extensive use. Basal media with 0.05% SDS as the sole carbon source was used to isolate bacteria that can utilize SDS. The isolates survived nitrogen-free medium and solubilized potassium and phosphate. Using 16S rRNA sequencing, *Enterobacter cloacae* strain MSK86 (OR136425) was identified. Stains-all dye was used to test the bacteria's SDS-utilizing capability. A 49% drop in SDS levels in the broth was observed after 7 days of 24-hour analysis. The bacteria exhibited tolerance to heavy metals like Cd (II), Ar (III), and Zn (II) at concentrations up to 2000 ppm, whereas they were susceptible to Cu (II), Cr (II), and Pb (II) at minimum concentrations of 200, 600, and 1000 ppm, respectively. The bacteria effectively reduced SDS levels in the laundry wash water. The treated water was reused for the irrigation of *Capsicum annum* L. and *Solanum lycopersicum* L. until the 45th day of growth. The plants' morphological and phytochemical properties were also analyzed. The potential of bacteria for SDS degradation and plant growth enhancement has been extensively explored independently; however, these traits have not been studied together in a single bacterial strain. In the present study, multifaceted *Enterobacter cloacae* MSK86 was isolated with these capabilities together, which may help in SDS remediation, making the water reusable for irrigation.

Keywords: SDS removal, Stains-all dye, heavy metal tolerance, laundry wastewater treatment, detergent pollution

INTRODUCTION

Detergents are one of the major causes of pollution in water bodies today **(Rai, 2006)**. According to pollution statistics, detergent consumption in India is 2.7 kg per capita per year, whereas in the USA it is 10kg per capita per year **(Senapati, 2021)**. The use of detergent is inevitable even though it causes chemical pollution in the water bodies. The untreated water enters the water streams and causes pollution, which may be detrimental to aquatic life. The lakes in the urban cities of India are witnessing seasonal foaming, which sometimes catches fire also **(Das *et al*., 2023)**. Most detergents are biodegradable as microbes produce enzymes to degrade most of the chemicals by utilizing them as their carbon source. The primary pollutant from household greywater is anionic detergent in the form of linear alkyl sulfonate (LAS) called sodium dodecyl sulfate (SDS) **(Jena *et al*., 2023)**. SDS is a biodegradable anionic detergent; several microbes were revealed for its SDS degrading capability. Although SDS is harmless in small amounts, it accumulates and becomes hazardous to aquatic life, soil microbiota, and insect communities. Bacterial bioremediation is an eco-friendly, sustainable, and cost-effective method over chemical-based sewage treatment **(Kuppan *et al*., 2024)**. Household greywaters such as laundry wash water (LWW), dishwashing water (DWW), and bathing water (BW) can be reused more easily than industrial effluents because we are much more aware of the water pollutants in the household cleaning agents. Therefore, these greywaters can be reused for various household purposes; one among them is watering plants (Patil *et al*., 2022). Some *Pseudomonas* **(Ellis*et al*., 2002; Jovcic *et al*., 2010; Shahbazi R, 2013; Venkatesh, 2013)**, *Bacillus* **(Singh *et al*., 1998)**, and *Klebsiella* **(Goodnow & Harrison, 1972a; Masdor *et al*., 2015)** species are reported to have SDS degradation potential. These bacteria produce alkyl sulfatase enzymes to break down SDS into dodecanol, an environmentally safe compound. Different microorganisms have different degrading potentials **(Furmanczyk *et al*., 2018)**.
Agriculture is one of the most water-intensive sectors, according to the Food and Agriculture Organization of the United Nations (2024). Around the world, 72 percent of all surface and groundwater withdrawals are used for crops, mainly irrigation, emphasizing water recycling for irrigation **(Ingrao *et al*., 2023)**. In this research, we made an attempt to find out a solution to these two problems simultaneously by isolating a bacterium with dual capabilities of SDS remediation and also promoting plant growth promotion. Plant growth-promoting bacteria are cleaner alternatives to chemical fertilizers and pesticides, as they reduce environmental pollution and enhance agricultural yields **(de Andrade *et al*., 2023)**. Numerous plant growth-promoting bacteria are prevalent, and many have been isolated and assessed for their growth-enhancing capabilities that promote plant growth and development by many mechanisms, including nitrogen fixation in the rhizosphere and synthesizing phytohormones such as auxins, cytokinins, and gibberellins, which enhance the uptake of nutrients from the environment **(Moreira& Bomfim, 2024)**. *Enterobacter* species are known for their plant growth-promoting traits, but a few reports are available on SDS **(Rahman *et al*., 2016)**. From the genus *Enterobacter*, *E. radicincitans*, *E. cloacae* subsp. cloacae, *E. asburiae*, *E. ludwigii*, *and E. gergoviae* have been documented to exhibit plant growth-promoting characteristics, including nutrient mobilization, siderophore synthesis, ACC-deaminase activity, and phytohormone production **(Jha *et al*., 2011)**. *Enterobacter cloacae* subsp. dissolvens MDSR9 isolated from the soybean rhizosphere exhibited IAA production, siderophore synthesis, ammonia generation, phytate mineralization, and phosphate, potassium, and zinc solubilization **(Ramesh *et al*., 2014)**. In recent years, biofortification techniques have become increasingly popular among researchers to boost the efficiency of sewage treatment systems **(Jangra *et al*., 2024)** .
This investigation on SDS remediation from LWW by *Enterobacter cloacae* MSK86, possessing plant-growth-promoting attributes, illustrates a sustainable method for repurposing detergent-contaminated water for agricultural usage. This approach prevents the influx of substantial amounts of SDS into lakes and rivers, thus safeguarding aquatic organisms and humans from numerous disease outbreaks caused by environmental contamination. Enriching water with diverse, multifaceted bacteria in water treatments diminishes the need for chemical fertilizers also by benefiting the soil communities that enhance soil fertility. Biofortification techniques have been increasingly popular among researchers to boost sewage treatment systems' efficiency in recent years. This study seeks to preserve the planet's water supplies by reusing it.

MATERIALS AND METHODS

Reagents and media composition

All media compositions are provided in grams per liter (g/L). SDS-BM agar composition: KH_2PO_4 3.5 g, K_2HPO_4 1.5 g, NH_4Cl 0.5 g, NaCl 0.5 g, Na_2SO_4 0.14 g, $MgCl_2 \cdot 6H_2O$ 0.15 g, SDS 0.5 g; Agar 20 g; pH 7.1. Jensen's agar (composition of HiMedia M710), Pikovskayas agar (HiMedia M520 composition), Aleksandrow agar (HiMedia M1996 composition), Stains-all dye (ChemPure), and Chrome azurol S (CAS) agar.

Isolation of SDS-utilizing bacteria

Soil and water samples were taken from various regions of South India. The SDS-tolerant bacteria were isolated utilizing basal media with SDS as the sole carbon source. One gram of soil was mixed vigorously in 10 mL of sterile distilled water. After being allowed to settle for 5 minutes, 100 µL of the solution was plated on SDS agar and incubated for 72 hours at 37 °C **(Furmanczyk *et al*., 2017)**. The colonies were subcultured onto SDS agar plates until pure bacterial colonies were obtained. The pure cultures were preserved in a 25% glycerol stock at -20 °C.

Screening for nitrogen fixation

Nitrogen-fixing free-living bacteria can fix atmospheric nitrogen for cellular protein synthesis and thrive on nitrogen-free media. Jensen's medium is the preferred medium for detecting and cultivating nitrogen-fixing bacteria **(Jensen, 1942)**. The chosen bacteria were inoculated into Jensen's agar plates and cultured for 7 to 12 days at 37 °C. The absence of nitrogen in the agar plates permits the growth solely of nitrogen-fixing organisms.

Potassium and phosphorus solubilization

The nitrogen-fixing bacteria obtained were further screened for potassium and phosphate solubilization. The potassium and phosphorus solubilization were assessed on Aleksandrow and Pikovskayas agar plates. Aleksandrow agar plates containing 0.004% phenol red pH indicator were utilized to determine potassium solubilization by the microorganisms **(Meena *et al*., 2015; Sun *et al*., 2020)**. Phosphate solubilization was assessed on Pikovskayas agar with a modification that included the addition of 0.004% bromocresol green **(Edi-Premono, 1996; Pikovskaya, 1948)**. The bacteria produce byproducts that change the pH of the agar, which can be made more visible by changing the indicator's color. The bacteria were spot-inoculated onto the plates and incubated at 37 °C for 24 hours, after which the zone surrounding the colony was measured using an antibiotic zone scale. The potassium and phosphate solubilization indices (SI) were determined by dividing the entire diameter of the halo zone by the diameter of the colony **(Watanabe, 1965)**.

Characterization, identification of the bacteria by 16S rRNA sequencing, and construction of phylogenetic tree

Colony characteristics and biochemical analyses were carried out, including gram staining, indole production, catalase activity, methyl red-Voges Proskauer (MRVP) test, Simmons citrate utilization, and oxidative fermentative tests.
Bacterial identification was carried out by 16S rRNA sequencing. The NucleoSpin® Tissue Kit extracted bacterium genomic DNA. Agarose gel electrophoresis verified DNA purity and quantity. An Applied Biosystems GeneAmp PCR System 9700 thermal cycler amplified the DNA. The 16S-RS-F (CAGGCCTAACACATGCAAGTC) and 16S rRNA primers targeted 16S rRNA. PCR products were analyzed on 1.2% agarose gels with 0.5X TBE buffer and 0.5 µg/ml ethidium bromide. ExoSAP-IT (USB) uses two hydrolytic enzymes, Exonuclease I and Shrimp Alkaline Phosphatase (SAP), to eliminate undesired primers and dNTPs from the PCR product mixture without impacting subsequent processes. Mix 0.5 µl of ExoSAP-IT with 5 µl of PCR product, incubate at 37°C for 15 minutes, and inactivate at 85°C for 5 minutes. PCR thermal cyclers and BigDye Terminator v3.1 Cycle Sequencing Kits were utilized for sequencing. Sequence quality was assessed using Sequence Scanner Software version 1. Sequence alignment and editing were done with Geneious Pro v5.1.

Quantification of the SDS in the medium

The inoculum for the SDS degradation analysis was prepared by subculturing 150 µL of a fresh overnight culture into 15 mL of LB broth. The culture was centrifuged at 8000 rpm for 5 minutes, and the bacterial cell pellet was resuspended in 500 µl of SDS broth and added to 100 mL of SDS basal media. The uninoculated SDS basal media containing 0.05% SDS served as a control. The flasks were incubated for 6 days at 28°C (± 2) on a rotary shaker at 120 rpm. Every 24 hours, 1 mL of culture was extracted and centrifuged at 10,000 rpm for 10 minutes at 24 °C. The cell-free supernatant was utilized to measure SDS concentration using the Stains-all method. In summary, 2 mL of the Stains-all intermediate solution was combined with 20 µL of the cell-free supernatant (1:100 µL) in a microfuge tube and thoroughly mixed. The absorbance was measured at 440 nm, and the decrease in SDS concentration was calculated with the help of an SDS calibration graph **(Furmanczyk *et al*., 2017; Rupprecht *et al*., 2015; Rusconi *et al*., 2001)**.

Heavy metal tolerance by well-diffusion method

The heavy metal toxicity of Cu (II), Pb (II), Zn (II), Cr (II), Cd (II), and Ar (III) against the bacteria was assessed using the standard well-diffusion method with various concentrations such as 200, 400, 600, 800, 1000, 1500, and 2000 ppm. Using a cork borer of 6mm diameter, wells were made in an LB agar plate with bacteria spread, and 200 µL of each concentration of heavy metal solutions was introduced into the well. After 24 hours at 37 °C, the plates exhibited a definite zone of inhibition, indicating the bacteria's susceptibility to the specific heavy metal, and it was noted **(Parmar *et al*., 2020)**.

Salt tolerance efficiency test

The bacteria were assessed for salt tolerance efficacy at different concentrations of NaCl. The LB agar was formulated with five concentrations of NaCl: 2%, 4%, 6%, 8%, and 10%. The bacteria were inoculated on the plates and incubated for 24 hours at 37 °C. The growth was checked after incubation **(Lanyi, 1979; Radhakrishnan & Krishnasamy, 2024)**.

Bacterial treatment of the laundry wash water

Domestic LWW was used for the study. LWW was autoclaved and tested for the pH, TDS, electrical conductivity, carbonates and bicarbonates, chloride, sodium, heavy metals (lead, cadmium, and chromium), and the SDS content. The bacterial inoculum was prepared as given before in Section 2.6. The collected cells were resuspended in 500 µL sterile LWW and added to 500 mL autoclaved LWW. Flasks were incubated at 28 °C (±2) with 120 rpm shaking. The concentration of SDS in LWW was tested at the 0^{th}, 24^{th}, and 48th h of incubation using Stains-all dye.

Pot studies on *Solanum lycopersicum* L. and *Capsicum annum* L. seedlings

The Arka variety of *Solanum lycopersicum* L. and *Capsicum annum* L. seeds were obtained from the Indian Institute of Horticulture (IIHR), Bangalore, India. The potting mix was made by combining soil, cocopeat, and compost in a ratio of 4:2:1 and autoclaved at 121 °C. The potting mix was analyzed for pH, electrical conductivity, organic carbon, macro- and micronutrients, and heavy metals **(Shakoor, 2018)**. The outcomes were deemed optimal for cultivation. Seeds underwent surface sterilization and were sown in a seedling tray with distilled water applied until the 21st day. Upon completion of the germination stage, seedlings were relocated to plastic pots filled with autoclaved potting mix and irrigated with bacteria-treated greywater (test) and distilled water (positive control). All these botanical investigations were performed in triplicate.

Morphological analysis of the seedlings

The plants were irrigated with treated-enriched water. On the 45^{th} day, the seedlings were harvested and washed with tap water to remove dirt and soil. Morphological features such as shoot length, root length, and number of leaves were noted, followed by phytochemical analysis, such as total sugar, protein, proline, chlorophyll, phenols, and flavonoids.

Phytochemical analysis of the seedlings

The fresh leaves of the harvested seedlings were used to test sugar, protein, proline, and chlorophyll, and plant extract was used to check for phenols and flavonoids. The phenol-sulfuric acid method was used to estimate the total sugar. Lowry's method was used to estimate the seedlings' total protein content **(Lowry *et al*., 1951)**. Arnon's method used an equation to estimate the leaves' total chlorophyll **(Arnon, 1949)**.

Total Chlorophyll (mg/g) = 20.2 (OD645) + 8.02 (OD663) x (V/(1000 x wt))

Estimating total proline was performed based on the protocol of Shabnam *et al*., a modified version of Betes's method **(Shabnam *et al*., 2016)**.
The harvested plants were shade-dried and subsequently ground using an electric grinder. The powder was then subjected to extraction using 99.8% methanol by the cold maceration process. The liquid extract obtained was filtered using Whatman No. 1 filter paper. The filtrate was then kept in a rotating shaker to yield the dried plant extract in its raw state. It was then resuspended in methanol at a concentration of 10 mg/mL and kept as stock solutions in a refrigerator at 4 °C for future use **(Mehmood *et al*., 2022)**.
The total flavonoid and phenolic content in the plant extract at a concentration of 1 mg/mL was determined using the aluminum chloride method and the Folin-Ciocalteu method, respectively **(Ahmad *et al*., 2018; Saeidnia *et al*., 2011)**.

Statistical analysis

IBM SPSS Statistics Version 25 and Microsoft Excel were used for the statistical studies. The seedlings' phytochemical measurement was done in triplicate using IBM-SPSS software and the one-way ANOVA function in Microsoft Excel. The graphs were created using Microsoft Excel. The standard error was used to express the results.

RESULTS AND DISCUSSION

Isolation of SDS-tolerant nitrogen fixers

Plating nine samples on SDS agar plates yielded the pure culture of 34 bacterial isolates (Fig. 1(a)). The bacteria were evaluated for nitrogen-fixing capability using nitrogen-free Jensen's media, and the bacterial isolate (BI 9) isolated from garden soil at the GKVK campus in Bengaluru, India (13°05'17.1"N 77°34'27.3"E), was observed to have luxurious growth on Jensen's media (Fig. 1 (b)).

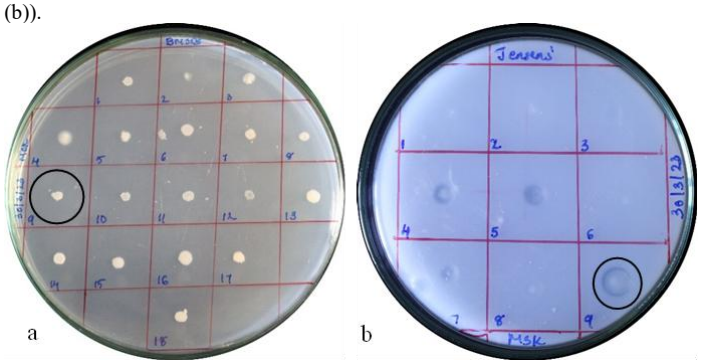

Figure 1 (a) The petri plates show the growth of 34 distinct pure bacterial isolates on BM-SDS agar. **(b)** The nitrogen-free Jensen's agar plates exhibit bacterial growth that is capable of fixing atmospheric nitrogen. BI 9 appeared to be luxuriant on Jensen's agar.

Potassium and phosphate solubilization

The bacteria demonstrated the ability to solubilize potassium and phosphorus, as depicted in Figures 2 (a) and 2 (b). The color surrounding the bacterial colony changed to yellow, indicating the solubilization of potassium and phosphorus. The potassium solubilizing index (KSI) and phosphorus solubilizing index (PSI) of BI 9 are expressed in the mean with its standard deviation. The KSI and PSI are 4.78 mm (± 0.43) and 3.44 mm (± 0.19).

Pseudomonas and *Bacillus* species are the most frequently referenced microorganisms in bioremediation, plant growth stimulation, and other beneficial applications for humanity **(Goodnow & Harrison, 1972b; Pirttilä *et al*., 2021)**. The members of the *Pseudomonas* genus can endure prolonged periods under adverse environments as they can degrade anionic surfactants, and their members are known for their plant growth-promoting activities. *P. aeruginosa* can degrade SDS and efficiently boost plant development through mechanisms such as phosphate solubilization, synthesis of indole acetic acid, and siderophore formation **(Vadnerker *et al*., 2018)**. *Bacillus cereus* was reported to break down SDS to dodecanol via the enzyme alkyl sulfatase without an organic carbon source **(Singh *et al*., 1998)**. *Azotobacter chroococcum* and *A. vinelandii* have demonstrated the ability to degrade anionic detergents and are globally utilized as nitrogen fixers **(Goodnow & Harrison, 1972b; Pirttilä *et al*., 2021)**.

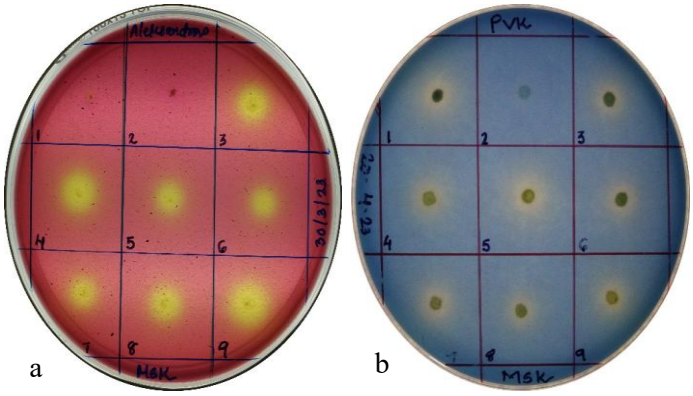

Figure 1 (a) The Aleksandrow agar was modified by adding 0.004% phenol red, indicating the potassium solubilizing yellow zone around the colony. (b) The Pikovskaya agar was modified with 0.004% bromocresol green, showing phosphorous solubilization zones around the colony.

Characterization and molecular identification of the bacteria

Colony morphology and biochemical characteristics (Fig. 3) of the bacteria were studied, and both bacterial colonies exhibited distinct characteristics beneficial for preliminary identification. The characteristics of the bacteria are given in Table 3.

Table 3 Colony morphology and biochemical characteristics of BI 9

Serial No.	Colony morphology & Biochemical characteristics	BI 9
1	**Shape**	Irregular
2	**Margin**	Lobate
3	**Elevation**	Umbonate
4	**Size**	Medium
5	**Color**	White
6	**Gram staining**	Gram-negative
7	**Indole test**	Negative
8	**Catalase activity**	Positive
9	**MRVP**	$MR^{-}VP^{+}$
10	**Citrate utilization**	Positive
11	**Oxidative fermentative test**	Fermentative

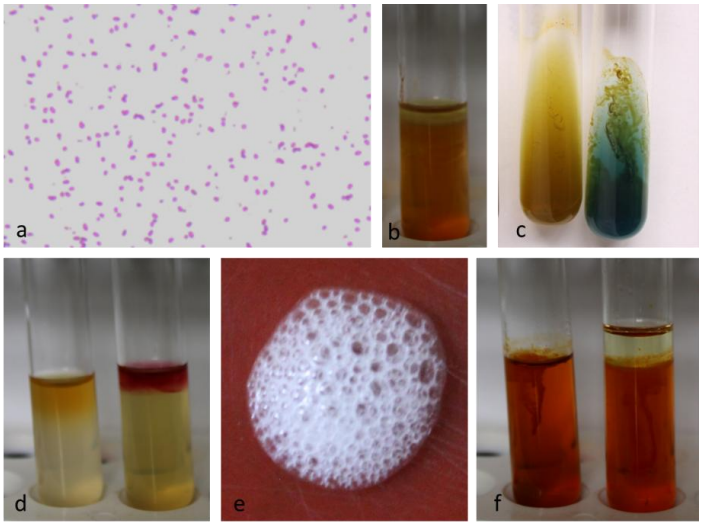

Figure 3 Biochemical characterization of bacteria. (a) Gram staining, (b) Indole test, (c) Citrate utilization, (d) MR VP (e) Catalase test, (f) Oxidative-fermentative test

Molecular identification by 16S rRNA sequencing followed by the BLAST analysis of the obtained gene sequence of the bacterium showed 100% similarity with strains of *Enterobacter cloacae*. The sequence was submitted to the NCBI databases with an accession number OR136425. Based on the high sequence similarity, the isolate was identified and named *Enterobacter cloacae* MSK86, and a phylogenetic tree was constructed using MEGA 11 in Fig. 4.

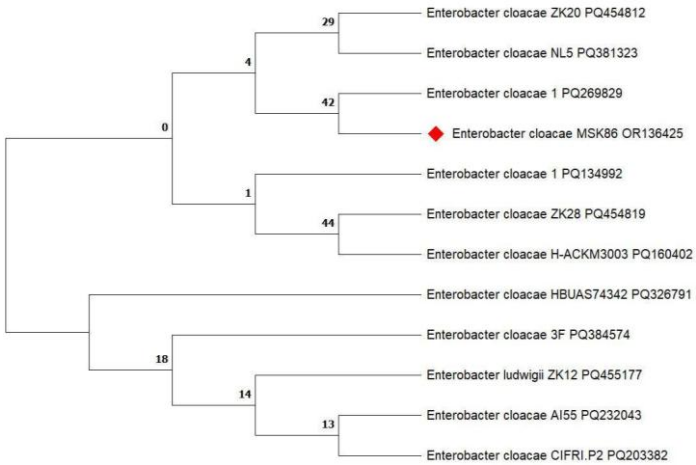

Figure 4 The phylogenetic tree depicts the newly identified E. cloacae MSK86, closely related to the many existing E. cloacae in the GenBank NCBI nucleotide database. The tree was constructed using MEGA11, a maximum likelihood method.

Quantification of SDS

The potential of bacteria to decrease the content of SDS in the SDS broth was assessed utilizing the Stains-all dye. The set concentration of SDS in the broth was 0.05%, regarded as 100%. The decrease in SDS concentration by *E. cloacae*

MSK86 was checked every 24 h until the 7th day. There have been fewer studies on SDS removal by *Enterobacter* spp. *Enterobacter* sp. strain Neni-13 has been reported for SDS remediation, which removed 800 mg/L in 12 days (Rahman *et al*., 2016). This may be the first study on SDS removal by *Enterobacter cloacae*. In this study, *Enterobacter cloacae* removed 49% from 0.5 g/L SDS on the 7th day. The SDS content of LWW before bacterial treatment was 0.296 g/L (± 0.0170), which decreased to 0.15 g/L (± 0.008). 49% of SDS removal was noted after 2 days of incubation. Various factors may affect the SDS removal by bacteria from LWW. First, LWW is an unknown formulation, and the bacteria have the chance of getting carbon from other components in LWW. Most bacteria are in the stationary phase during the second day of incubation. As the bacteria are present in a stressful environment, it is crucial to keep their viability for expressing plant growth-promoting traits so that bacteria can be transferred to soil by irrigating the fortified water for cultivation.

Heavy metal and salt tolerance of *Enterobacter cloacae* MSK86

The effect of Cd (II), Cu (II), Cr (II), Zn (II), Pb (II), and Ar (III) at concentrations ranging from 200 to 2000 ppm on bacterial growth is shown in Figure 4. The bacteria were resistant to Cd (II), Ar (III), and Zn (II) at concentrations up to 2000 ppm. However, the bacteria were susceptible to Cu (II) with minimum concentration of 200 ppm, Cr (III) at 600 ppm, and Pb (II) at 1000 ppm. The bacteria tolerated up to 6% salt concentrations, but their growth was reduced with the increasing concentration of NaCl, and no growth was observed on the plate with 10% NaCl (Fig. 5).

Using bacteria for bioremediation and enrichment of greywater results in the entry of bacteria into the soil, where numerous elements, including heavy metals and soil salinity, influence bacterial growth and may hinder anticipated outcomes. Using bacteria with diverse stress tolerance capabilities is preferable for bioremediation studies **(Shindee *et al*., 2023)**. Heavy metals in the soil may impede microbial activity and adversely affect crop quality **(Ilao *et al*., 2014)**. Although heavy metal contamination is not ubiquitous in all soil, trace amounts of lead, copper, cadmium, chromium, nickel, zinc, and others may be present **(Xiang *et al*., 2021)**. Mohan reports that around 718 districts in India possess groundwater contaminated with arsenic, cadmium, chromium, and lead **(Mohan, 2018)**. The Ministry of Environment, Forest and Climate Change (MoEF & CC) has identified 320 locations in India with a significant risk of contamination from heavy metals (Cr, Pb, Hg, As, and Cu) and pesticides Detergents are one of the major causes of pollution in water bodies today **(Rai, 2006)**. According to pollution statistics, detergent consumption in India is 2.7 kg per capita per year, whereas in the USA it is 10kg per capita per year **(Senapati, 2021)**. The use of detergent is inevitable even though it causes chemical pollution in the water bodies. The untreated water enters the water streams and causes pollution, which may be detrimental to aquatic life. The lakes in the urban cities of India are witnessing seasonal foaming, which sometimes catches fire also **(Das *et al*., 2023)**. Most detergents are biodegradable as microbes produce enzymes to degrade most of the chemicals by utilizing them as their carbon source. The primary pollutant from household greywater is anionic detergent in the form of linear alkyl sulfonate (LAS) called sodium dodecyl sulfate (SDS) **(Jena *et al*., 2023)**. SDS is a biodegradable anionic detergent; several microbes were revealed for its SDS degrading capability. Although SDS is harmless in small amounts, it accumulates and becomes hazardous to aquatic life, soil microbiota, and insect communities. Bacterial bioremediation is an eco-friendly, sustainable, and cost-effective method over chemical-based sewage treatment **(Kuppan *et al*., 2024)**. Household greywaters such as laundry wash water (LWW), dishwashing water (DWW), and bathing water (BW) can be reused more easily than industrial effluents because we are much more aware of the water pollutants in the household cleaning agents. Therefore, these greywaters can be reused for various household purposes; one among them is watering plants (Patil *et al*., 2022). Some *Pseudomonas* **(Ellis*et al*., 2002; Jovcic *et al*., 2010; Shahbazi R, 2013; Venkatesh, 2013)**, *Bacillus* **(Singh *et al*., 1998)**, and *Klebsiella* **(Goodnow & Harrison, 1972a; Masdor *et al*., 2015)** species are reported to have SDS degradation potential. These bacteria produce alkyl sulfatase enzymes to break down SDS into dodecanol, an environmentally safe compound. Different microorganisms have different degrading potentials **(Furmanczyk *et al*., 2018)**.

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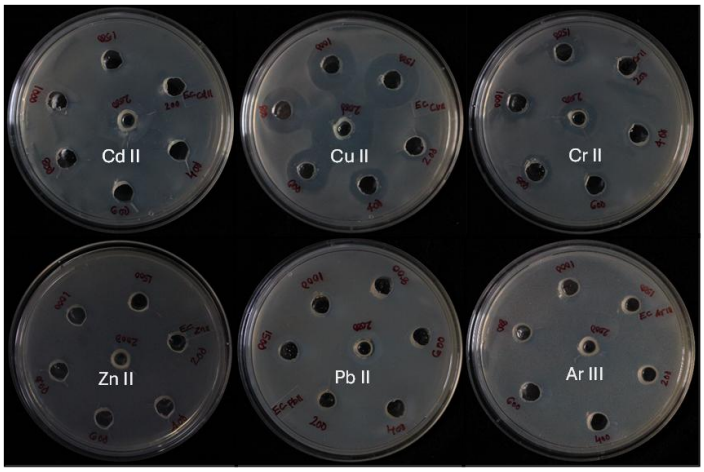

Figure 4 The plates on which *Enterobacter cloacae* MSK86 grows against various metal cations by the well-diffusion method.

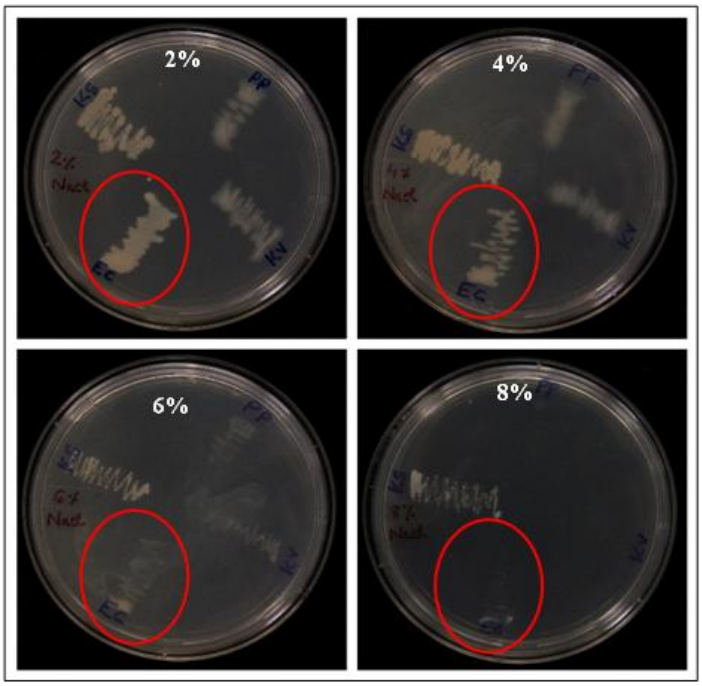

Figure 5 The plates depict the salinity tolerance of *Enterobacter cloacae* MSK86

Salinity is a significant abiotic stress, affecting 3% of the Earth's terrestrial area, which is one of the significant factors contributing to stagnant agricultural growth. Of the 147 million hectares in India, roughly 23 million are impacted by soil deterioration, primarily due to salt, alkalinity, or acidification (P. Kumar & Sharma, 2020). Using salinity-tolerant plant growth-promoting microorganisms in

agriculture is an environmentally sustainable solution to this issue (Dodd & Perez-Alfocea, 2012). Literature has shown that certain *Enterobacter* species, including *E. cloacae* PM23, *E. cloacae* KBPD, *E. cloacae* HSNJ4, *E. cloacae* MU-1, and *E. cloacae* Rs-35, promote plant growth even in high salinity conditions (Ali *et al*., 2022; Bhise *et al*., 2017; Hua *et al*., 2010; Li *et al*., 2017; Yue *et al*., 2023). The SDS-tolerant *Enterobacter cloacae* strain isolated in this study, which has plant growth-promoting traits, can be considered a candidate for bioremediation of heavy metals and helping plants grow even in salinity environments.

Pot studies on *Capsicum annum* L. and *Solanum lycopersicum* L.

In this study, eight plant parameters were checked by comparing the bacterial-treated plants with the untreated ones. The morphology of the plants, such as shoot and root lengths, was checked, as depicted in figure 6. All the values were calculated in mg/g and compared with the positive control.

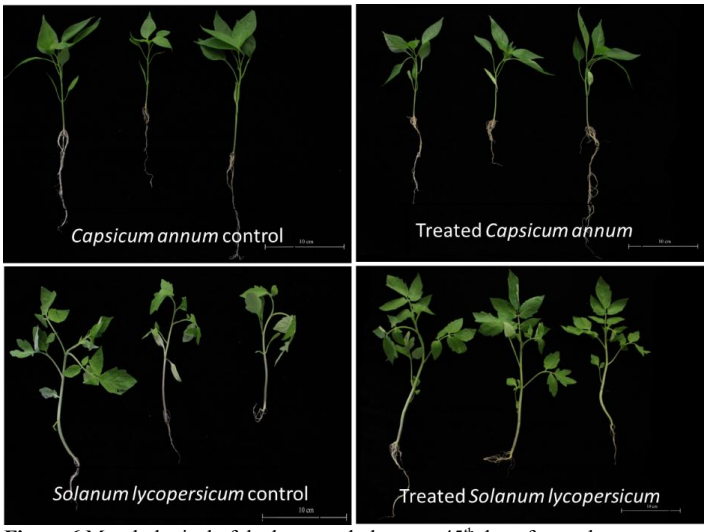

Figure 6 Morphological of the harvested plants on 45th day of growth

Table 1 Morphological and phytochemical analysis of *Capsicum annum* L. and *Solanum lycopersicum* L. The results were represented as the mean of triplicates with standard error. The DMRT analysis indicates the values with the same alphabets are not significantly different at P≤0.05.

Seedlings	Treatments	Shoot length (cm)	Root length (cm)	Sugar (mg/g)	Protein (mg/g)	Chlorophyll (mg/g)	Proline (mg/g)	Flavonoid (mg/g)	Phenol (mg/g)
***Capsicum annum* L.**	Control	9.67 ± 0.33b	10.0 ± 0.58b	56.39 ±0.30a	7.14 ± 0.03a	2.38 ± 0.04c	6.47 ± 0.02c	0.52 ± 0.05b	3.1 ± 0.52b
	E. cloacae MSK86	8.17 ± 0.17c	13.5 ± 0.29a	46.53 ±0.25b	8.73 ± 0.27c	4.25 ± 0.03a	8.50 ± 0.23a	0.45 ± 0.07b	2.64 ± 0.18b
***Solanum lycopersicum* L.**	Control	9.00 ± 0.58b	4.0 ± 0.58c	43.75 ±0.32d	8.18 ± 0.13d	1.98 ± 0.04d	7.58 ± 0.06b	1.10 ± 0.03a	9.02 ± 0.26a
	E. cloacae MSK86	16.50 ± 0.50a10	13.0 ± 0.58a	49.51 ± 0.25c	8.22 ± 0.12b	3.66 ± 0.03b	8.20 ± 0.39b	1.18 ± 0.01a	9.73± 0.03a

After irrigating the plant with *E. cloacae* MSK86-treated greywater, root length, protein, chlorophyll, and proline significantly increased in both plant varieties. The root length of *Capsicum annum* seedlings increased 1.35 times, protein 1.2 times, chlorophyll 1.8 times, and proline content 1.3 times compared with the control plants. Whereas *Solanum lycopersicum* showed significant increases in all eight parameters analyzed. The root length and shoot length increased 3 and 1.8 times, respectively. The phytochemical parameters such as chlorophyll, sugar, protein, and proline increased 1.9, 1.13, 1.1, and 1.1 times compared to control plants. In the present study, *E. cloacae* MSK86 is highly advantageous for the growth enhancement of *Solanum lycopersicum*; however, in *Capsicum annuum*, the bacterium did not exhibit a favorable influence on all eight criteria assessed. *Enterobacter* sp. DBA51 root inoculations increased plant height by 20% and root biomass by 40% in tomato (*Solanum lycopersicum* L.); however, in tobacco (*Nicotiana tabacum* L.), only root biomass (27%) increased (Ortega-Ortega *et al*., 2024). Briefly, the impact of *Enterobacter* species, on plant growth can be beneficial or detrimental, depending on the strain and plant species involved. In this study, we employed bacterially treated water to irrigate plants, a novel approach. The multifaceted Enterobacter cloacae isolated from the garden can utilize SDS from the detergent-polluted water and enrich the water with properties that promote plant growth. This investigation was trying to address water pollution caused by our household cleaning agents. Reducing, recycling, and reusing water is the best way to cope with water scarcity. This solution addresses pollution and sustainable farming by combining environmental purification with agricultural development. It is a unique approach that combines SDS utilization and plant growth improvement with a single bacterial strain. The findings enable bio-based wastewater treatment and water recycling for sustainable agriculture and ecological equilibrium.

CONCLUSION

Enterobacter cloacae strain MSK86, a multifaceted bacterium, is capable of SDS bioremediation and plant growth promotion and can be considered a candidate for biofertilizer with proper biosafety and pathogenicity checks. Bacterial enrichment in laundry wash water was made the water reusable for irrigation purposes. The bacteria were also tolerant to heavy metals such as Cd (II), Ar (III), and Zn (II) up to 2000 ppm. The pot studies showed that *E. cloacae* MSK86 can also enhance plant growth. Recognizing and employing diverse bacteria is essential for tackling environmental issues and advancing sustainable agriculture. It offers a comprehensive strategy for improving plant growth, remediating pollutants, reducing the use of chemical fertilizers, and minimizing the ecological impact of agricultural activities.

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CRediT authorship contribution statement Mittu Koshy: conceptualization, methodology, investigation, data curation, and writing the original manuscript.

Biljo V. Joseph: conceptualization, data analysis, supervision, reviewing, and manuscript editing.

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

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