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Selenium Biotransformation By Bacteria Isolated From Lahore And Exploring Their Biochemical Properties

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ABSTRACT

Background: Selenium is an important micronutrient for living organisms. However, its higher concentrations can be toxic to ecosystems due to its high bioavailability. In biogeochemical cycles, selenite is converted into elemental selenium by microorganisms. These processes can help depollute the environment and promote eco-friendly practices. The purpose of this study was to examine the distribution, diversity, and potential of selenite-reducing bacteria from samples of Lahore, Pakistan.

Materials & Methodology: The experiment involved the collection of a total of 6 different water and soil samples from all over Lahore. These samples were processed within 24 hours. Samples were grown on nutrient agar supplemented with 400 µg/mL sodium selenite. Twenty selenite-reducing isolates were collected and further tested for morphological, biochemical, physiological, and plant growth-promoting characteristics. Biochemical tests included catalase, oxidase, starch hydrolysis, nitrate reduction, MR/VP, citrate, and urease. Functional traits like producing indole-3-acetic acid, ammonia, and phosphate solubilization were examined to check for plant growth-promoting potential. The bacterial response to antibiotics and resistance to heavy metals was also tested. Physiological responses were measured under varying pH, temperature, salinity, and selenite concentrations using optical density at 500 nm. The data were analyzed descriptively and comparatively.

Results: The results showed that all samples reduced selenite and formed red elemental selenium. Most of the isolates were positive for catalase, oxidase, and nitrate reduction. This showed strong metabolic and redox potential. Many isolates produced significant amounts of auxin and ammonia, though no phosphate solubilization activity was observed. Discrete patterns of antibiotic and multidrug resistance were recorded among strains. The best selenite reduction occurred at optimal pH and temperature.

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Conclusion: Significant ecological and metabolic versatility and the ability to promote plant growth were recorded in these bacteria native to Lahore. This indicates that they have great potential to be used for selenium bioremediation and sustainable agriculture practices.

Introduction

Selenium is an important trace element that plays a significant role in biological functions and ecological balance. It supports antioxidant systems and metabolism in organisms. Higher levels of selenium, especially as selenite and selenate, can cause environmental poisoning. [1] These forms are easily absorbed by organisms and enter the environment through farm runoff, industrial waste, and mining activities. Traditional pollution cleanup methods are expensive and harmful to the environment, prompting the need for other natural alternatives.

Microorganisms are crucial in selenium biogeochemical cycling. They use enzyme-based and non-enzyme-based methods to convert harmful selenite into safer elemental selenium. [2, 3]. This process helps clean polluted areas and produces selenium nanoparticles, which are valuable for the environment and agriculture.

Bacteria such as *Lactobacillus casei* and *Shewanella oneidensis* employ various methods to break down substances, including enzymes called reductases and glutathione reactions. [1, 4]

Environmental isolates such as *Enterobacter cloacae* and *Bacillus* species are strong candidates for selenium bioremediation and wastewater treatment. [5, 6] Moreover, microbial consortia present in complex systems demonstrate their ecological adaptability. [7] Several selenite reducers have also been shown to promote plant growth through phytohormone production and nutrient cycling, improving plant productivity under stress. [8, 9]

Despite these developments, there remain gaps in understanding the ecological interactions, functional diversity, and environmental adaptability of these bacteria, particularly in less-explored regions. Consequently, studying natural environmental microbes is important for developing long-term solutions to pollution and enhancing farming methods.

Novelty Statement

To the best of our knowledge, this study provides the first combined assessment of Lahore's diversity of selenite-reducing bacteria, combining biochemical characterization and plant growth-promoting potential.

Study Objectives

This study aims to isolate and screen selenite-reducing bacteria from different environmental samples collected from Lahore, Pakistan. Isolates were then characterized based on morphology, biochemical, and physiological properties. Phosphate solubilization, indole-3-acetic acid, and ammonia production were performed to evaluate plant growth promotion potential. Moreover, the strains' tolerance to varying environmental stress factors like pH, temperature, salinity, and selenite concentrations was also tested. All this was aimed at investigating their potential for application in selenium bioremediation and sustainable agriculture.

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Materials and Methods

All experiments were performed under aseptic conditions. Glassware, reagents, chemicals, and media were autoclaved for 20 minutes at 121°C under 15 psi pressure [10]. All of the reagents were prepared using autoclaved distilled water and handled carefully following laboratory safety protocols. Agars and broths were prepared according to traditional recipes and techniques by dissolving the required ingredients in 1 liter of distilled water at neutral pH and autoclaving before use. [11, 12]

Sample Collection and Processing

Four soil and two water samples were collected in total. They were collected from multiple locations across Lahore, Pakistan, including agricultural lands (samples A and D), industrial sites (samples E and F), a graveyard (sample B), and a botanical garden (sample C). Soil samples were collected from the top layer of soil about 3-5 inches deep using sterile gloves and placed in labeled sterile zip-lock bags. Water samples were collected in labeled sterile plastic bottles. All samples were processed within 24 to 48 hours to minimize changes in the microbial community's structural changes and cell viability [13].

Isolation of Selenite-Reducing Bacteria

For isolation, 1 g of soil or 1 mL of water was serially diluted in 9 mL of distilled water up to a 10^{-5} dilution. 100 µL of the 1st, 3^{rd,} and 5th dilutions were spread onto nutrient agar plates containing 400 µg/mL sodium selenite. [14]. Plates were incubated at 37 °C for 24-48 hours. Reddish colonies were picked with a sterile inoculating loop and purified through quadrant streaking on fresh nutrient agar plates [14,15].

Morphological Characterization

Following purification, bacterial isolates were examined for colony morphology (shape, elevation, and margin). Gram staining was done by standard staining procedures to separate Gram-positive and Gram-negative bacteria. Endospore staining was performed using the Schaeffer-Fulton method using a 7-day-old culture with malachite green and safranin for spore-forming strains. [16].

Biochemical Characterization

Catalase Activity

24-hour-old bacterial cultures were placed on a slide, and a few drops of 3% hydrogen peroxide were added. Immediate release of free oxygen bubbles was recorded as a positive reaction for catalase activity [16].

Oxidase Test

The oxidase test was performed using 1% tetramethyl-p-phenylenediamine. A strip of filter paper was placed on a sterile work area. A loopful of the test isolate was carefully spread out over it with a sterile loop. A few drops of reagent were then added. A positive test result was indicated by a color change to purple within 30 seconds. [16].

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Starch Hydrolysis Test

Bacterial isolates were aseptically inoculated onto starch agar plates. Then incubated at 37 °C for 24 hours. After incubation, the plates were flooded completely with iodine solution. If a halo formed along bacterial growth, the isolate was considered positive for starch hydrolysis [16].

Nitrate Reduction Test

Reagents for nitrate reduction were prepared using standard protocols (reagents A and B). Isolates were inoculated into test tubes containing 5 mL of autoclaved nitrate broth and incubated for 24 hours at 37 °C. After incubation, 5 to 6 drops of each sulfanilic acid (reagent A) and α-naphthylamine (reagent B) were added. The formation of red color indicated nitrite formation. If no color change was observed within 2 minutes, zinc dust was added. If a red color developed after zinc addition, it implied that nitrate reduction had not occurred. While the complete absence of color showed a reduction of nitrate to either nitrogenous gases or ammonium [16].

Methyl Red and Voges-Proskauer Tests (MR/VP)

For the methyl red test, isolates were inoculated into test tubes containing 5 mL autoclaved glucose phosphate broth and incubated at 37 °C for 24 hours. This was done in duplicate; therefore, for 20 isolates, a total of 40 test tubes were used. Following incubation, 5-6 drops of MR reagent were added to half of the inoculated test tubes (all 20 strains). A red color indicated positive results. For the Voges-Proskauer test, a few drops of Barritt's reagents A (α-naphthol) and B (40% KOH) were added to the other half of the inoculated test tubes after 48 hours of incubation. A pinkish to red color within 30 minutes indicated acetoin production and hence was interpreted as positive [16].

Citrate Utilization Test

Simon citrate agar was prepared and autoclaved according to standard protocol [37]. Test-tube slants were created, and each test tube was inoculated with one isolate. Then, the test tubes were incubated for 24-48 hours at 37°C. After incubation, the color change of the media from green to dark blue was recorded as a positive result. [13].

Urease Production Test

Urease activity was tested by streaking test strains on Christensen's urea agar slants. Cultures were incubated at 30 °C for 24-48 hours. Development of a pink coloration indicated ammonia production and hence a positive result [14].

Hydrogen Cyanide Production

Hydrogen cyanide production was detected by supplementing the nutrient agar with 4.4 g L^{-1} of glycine. After bacterial isolates were streaked, the plate's lid was fitted with filter paper soaked in picrate solution (picric acid and sodium carbonate). Plates were sealed and incubated at 37 °C for 48 to 72 hours. A reddish-brown color change of the paper indicated positive results for hydrogen cyanide production [15]. Positive isolates were then tested for quantitative analysis by eluting filter paper in distilled

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water and homogenizing. Optical density was measured at 500 nm.

Assessment of Plant Growth-Promoting Traits

Phosphate Solubilization Assay

Phosphate solubilization was assessed using Pikovskaya's agar, containing tricalcium phosphate as an insoluble phosphate source. Isolates were streaked singly on plates and incubated at 37 °C for 5-7 days. Formation of clear halos around the inoculum indicated positive results due to acid production [17].

Indole-3-Acetic Acid (IAA) Production

Isolates were cultured in test tubes containing 5 mL of nutrient broth. Each strain was inoculated in two test tubes, one with nutrient broth supplemented with 0.1% tryptophan and the other with nutrient broth without tryptophan. Test tubes were then incubated at 37 °C for 48-72 hours. After incubation, cultures were centrifuged at 5000 rpm for 5 minutes, and the supernatant was obtained in a separate test tube. A few drops of Salkowski reagent were added to all supernatants. A pink color development after 30 minutes of incubation at room temperature in the dark indicated positive results. The optical density was taken for this bacterial culture at 500 nm after incubation. [18].

Ammonia Production Test

Test tubes with 4-5 mL of peptone water were inoculated with bacteria and incubated at 37 °C for 48-72 hours. After incubation, a few drops of Nessler's reagent were added to the inoculated peptone water. Brown to yellow coloration indicated ammonia production [17].

Antibiotic Susceptibility Testing

The antibiotic sensitivity test was done by the Kirby-Bauer disk diffusion method on nutrient agar as recommended by the Clinical and Laboratory Standards Institute (CLSI). 24-hour-old cultures were spread evenly on agar plates with a sterile cotton swab. Ceftazidime, tetracycline, piperacillin, neomycin, and chloramphenicol disks were used. Discs were placed on the surface of the inoculated petri plates and incubated at 37 °C for 24 hours. After incubation, zones of inhibition were measured and interpreted as per CLSI benchmarks [19].

Heavy Metal Resistance Assay

Heavy metal resistance profiles were performed by culturing isolates in nutrient agar plates supplemented with 400 µg/mL salt of each heavy metal. Salts were weighed at 0.5 g, and 1% stock solutions were prepared. Metals included lead, chromium, lithium, nickel, copper, cobalt, and mercury. Resistance or sensitivity was monitored after incubation for 48 hours at 37 °C [20].

Physiological Characterization of Bacterial Isolates

Bacterial isolates were evaluated for physiological tolerance in different pH, temperature, and selenite concentrations. The emphasis was on selenite reduction capacity as an indicator of metabolic activity [21, 22].

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Selenite Reduction at Different pH Levels

Nutrient broth was prepared and adjusted to three different pH ranges (pH 5, pH 7, and pH 9). This was done by using 1 M HCl or 1 M NaOH before sterilization. Media were supplemented with 400 µg mL^{-1} sodium selenite after autoclaving. 5 mL of this broth was added to test tubes, i.e., 21 tubes per pH, and inoculated. Cultures were centrifuged at 5000 rpm for 10 minutes after 48 hours of incubation at 37 °C. Supernatant was discarded, and pellets were washed with 1 M NaCl, recentrifuged, and then washed again with distilled water. Final pellets were resuspended in 2.5 mL of distilled water and vortexed. Selenite reduction was indicated by red color, and optical density was measured at 500 nm [22, 23].

Selenite Reduction at Different Temperatures

Autoclaved nutrient broth, 5 mL per test tube, was supplemented with 400 µg mL^{-1} sodium selenite, i.e., 21 test tubes per temperature. After inoculation, cultures were incubated at 28 °C, 37 °C, and 45 °C for 48 hours. After incubation, centrifugation, washing, and optical density measurement were performed as described above. [22, 24]

Selenite Reduction at Different Incubation Times

Sterile nutrient broth was supplemented with 400 µg mL^{-1} sodium selenite. 5 mL of it was dispensed in test tubes and inoculated with test strains and incubated at 37 °C, and selenite reduction activity was evaluated at 24, 36, and 48 hours. At each time interval, cultures were centrifuged at 5000 rpm for 10 minutes. Pellets were washed with 1 M NaCl, recentrifuged, washed, resuspended in 2.5 mL distilled water, and vortexed. Reduction of selenite was qualitatively indicated by red coloration due to the formation of elemental selenium and quantitatively determined by measuring optical density at 500 nm [25, 26].

Selenite Reduction at Different NaCl Concentrations

Nutrient broth containing 400 µg mL^{-1} sodium selenite was prepared with three NaCl concentrations (1%, 3%, and 5% w/v). The broth was then autoclaved and distributed into test tubes and inoculated with bacterial cultures. Tubes were incubated at 37 °C for 48 hours. After incubation, cultures were processed as described previously. [26, 27].

Selenite reduction at different sodium selenite concentrations

Nutrient broth had sodium selenite added to it at three levels (400, 800, and 1200 µg mL^{-1}). The broth was then sterilized in an autoclave, put into test tubes (20 for each concentration), and mixed with bacterial cultures. The tubes were kept at 37 °C for two days. After this time, the cultures were treated as mentioned above.

Results

Isolation of Selenite-Reducing Bacteria

From soil and water samples collected across Lahore, a total of 20 bacterial isolates named UA1-21 were obtained on nutrient agar containing 400 µg/mL sodium selenite. All of these isolates produced a red precipitate, indicative of elemental

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selenium formation (Figure 1). The highest number of selenite-reducing isolates was obtained from soil samples, indicating high selenium tolerance in that environment.

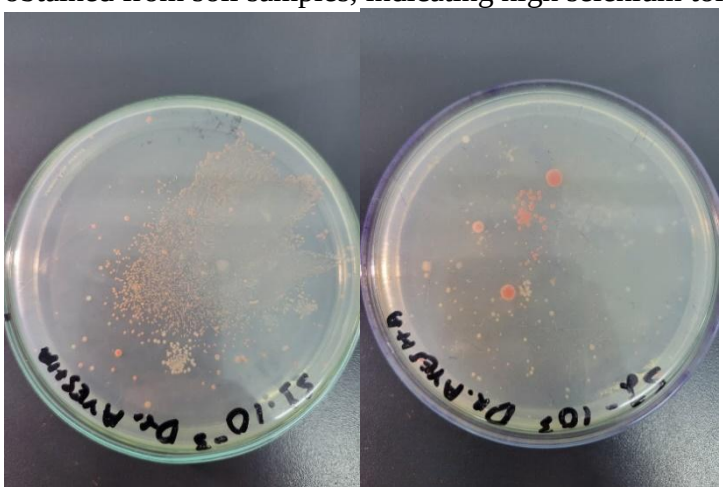

Figure 1: Representative colonies of selenite-reducing bacteria showing red-colored elemental selenium precipitation on nutrient agar plates.

Characterization Based on Cellular and Colony Morphology

Colonial morphology varied among isolates; most exhibited round colony morphology, which was observed in 14 isolates, while punctiform colonies were noted in 4 isolates. Filamentous and irregular colony shapes were each observed in isolation. Colony margins were predominantly complete, noted in 15 isolates, while undulate margins were observed in 3 isolates, and curled and lobulated margins were observed in one isolate each. Most of the isolates (18) showed flat elevations, while umbonate and raised elevations were recorded in one isolate each. Gram staining results showed that 13 isolates were Gram-positive, while 7 isolates were Gram-negative. Endospore formation was detected in 10 isolates, while spore formation was not observed in the remaining 10 isolates, consistent with their presumed Bacillus identity (Table 1).

Table 1: Morphological characteristics of selected selenite-reducing isolates.

Strain	Size	Shapes	Margins	Elevations	Texture	Color	Gram stain	Endospore stain
UA1	Small	Punctiform	Entire	Flat	Matte	White	Negative	Negative
UA2	Medium	Round	Entire	Flat	Matte	White	Negative	Negative
UA3	Medium	Round	Undulate	Flat	Slimy	creamy	Negative	Positive
UA4	Medium	Round	Entire	Flat	Slimy	White	Positive	Positive
UA5	Medium	Round	Entire	Flat	Matte	White	Negative	Negative
UA6	Medium	Round	Entire	Flat	Slimy	White	Positive	Negative
UA7	Medium	Round	Entire	Flat	Slimy	White	Positive	Negative
UA8	Small	Punctiform	Entire	Flat	Slimy	Creamy	Positive	Positive
UA9	Small	Round	Entire	Flat	Matte	White	Positive	Positive
UA10	Small	Round	Entire	Flat	Matte	White	Negative	Negative
UA11	Medium	Round	Entire	Flat	Slimy	Creamy	Positive	Negative

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UA12	Small	Round	Entire	Flat	Matte	White	Positive	Negative
UA13	Small	Round	Undulate	Flat	Matte	White	Negative	Positive
UA15	Medium	Round	Entire	Flat	Slimy	White	Positive	Positive
UA16	Small	Round	Entire	Flat	Matte	White	Positive	Positive
UA17	Medium	Round	Curled	Umbonate	Matte	Creamy	Positive	Positive
UA18	Large	Filamentous	Lobulated	Raised	Matte	White	Positive	Negative
UA19	Small	Punctiform	Entire	Flat	Matte	White	Positive	Positive
UA20	Small	Irregular	Undulate	Flat	Translucent	White	Negative	Negative
UA21	Small	Punctiform	Entire	Flat	Slimy	Creamy	Positive	Positive

Biochemical Characterization

Out of 20 bacterial isolates tested, 17 isolates were catalase positive, while 3 isolates were catalase negative. 16 positive and 4 negative isolates were observed in the oxidase test. Starch hydrolysis was observed in 16 isolates, while 4 isolates were negative for this test. Nitrate-reducing activity was detected in 19 isolates, with only 1 isolate showing a negative result. In the methyl red test, 15 isolates were positive, while 5 were negative, and Vogues-Proskauer was positive in only 5 strains. All strains were urease negative, but all except 3 were positive for the citrate test.

Table 2: Biochemical Profiling of Bacterial Isolates.

Isolates	**Catalase test**	**Oxidase test**	**Starch Hydrolysis**	**Nitrate Reduction**	**Methyl Red**	**Vogues Proskauer**	**Citrate Test**	**Urease test**
UA1	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Negative
UA2	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Negative
UA3	Positive	Positive	Positive	Positive	Negative	Negative	Negative	Negative
UA4	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Negative
UA5	Positive	Positive	Positive	Positive	Negative	Negative	Negative	Negative
UA6	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Negative
UA7	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Negative
UA8	Positive	Negative	Positive	Positive	Positive	Negative	Negative	Negative
UA9	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Negative
UA10	Positive	Positive	Positive	Positive	Negative	Negative	Positive	Negative
UA11	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Negative
UA12	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Negative
UA13	Negative	Positive	Negative	Positive	Positive	Positive	Positive	Negative
UA15	Negative	Positive	Positive	Positive	Positive	Negative	Positive	Negative
UA16	Negative	Positive	Negative	Positive	Positive	Positive	Positive	Negative
UA17	Positive	Negative	Negative	Positive	Negative	Negative	Positive	Negative
UA18	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Negative
UA19	Positive	Negative	Positive	Positive	Positive	Negative	Positive	Negative
UA20	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Negative
UA21	Positive	Positive	Positive	Negative	Positive	Negative	Positive	Negative

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Plant Growth-Promoting Traits

All isolates were evaluated for their ability to solubilize inorganic phosphate on Pikovskaya's agar. But all 20 isolates that lacked halo formation were considered negative. IAA production differed among isolates and was strongly influenced by the presence of tryptophan, as seen in Figure 2. Most isolates demonstrated the ability to produce ammonia in peptone water, as shown in Table 4. Antibiotic sensitivity patterns were variable among isolates when treated with tetracycline, ceftazidime, piperacillin, and chloramphenicol. Zones of inhibition were measured in mm and used to plot a bar chart as shown in Figure 3. All strains showed sensitivity to ceftazidime. Isolates displayed differing tolerance levels at 400 µg/mL of various heavy metal concentrations, as seen in the table. Several isolates exhibited multi-metal resistance, making them promising candidates for bioremediation (Table 3).

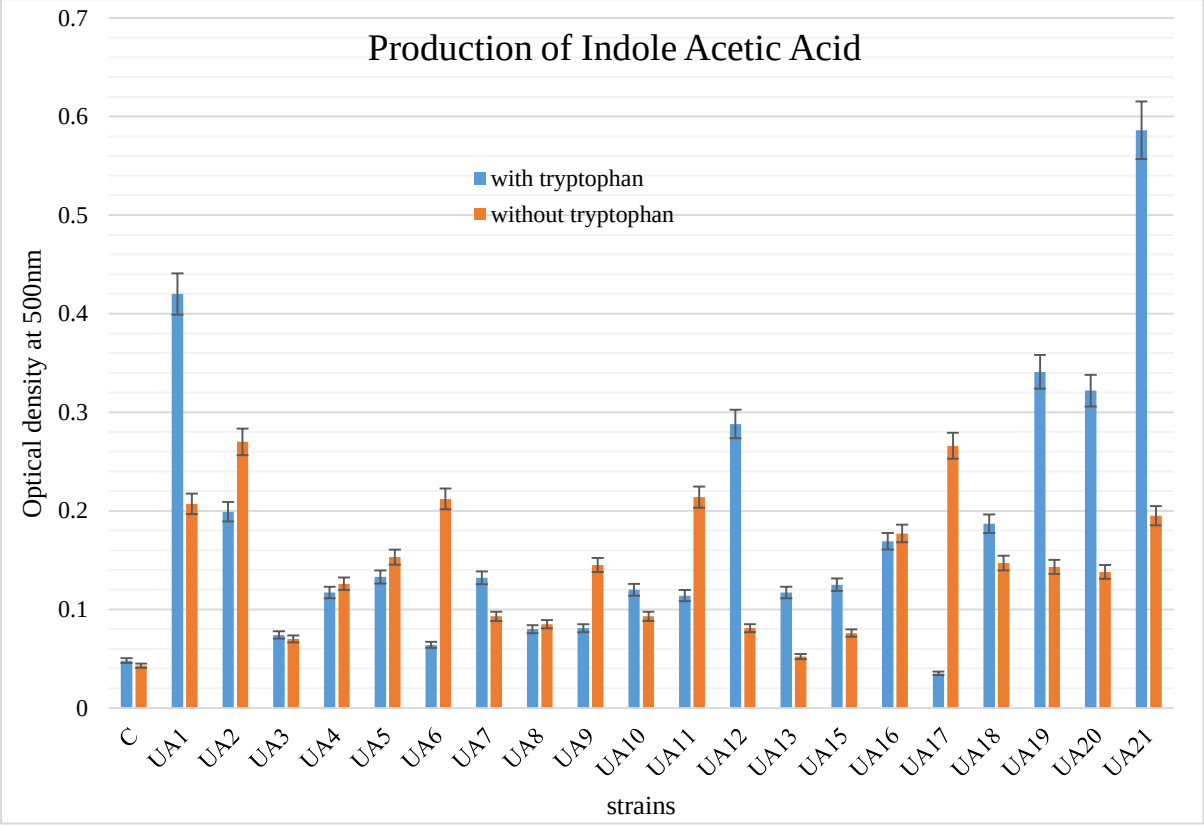

Figure 2: Indole-3-acetic acid (IAA) production: Optical density values representing IAA production by different bacterial strains (UA1-UA21) grown in media supplemented **with** and **without tryptophan.**

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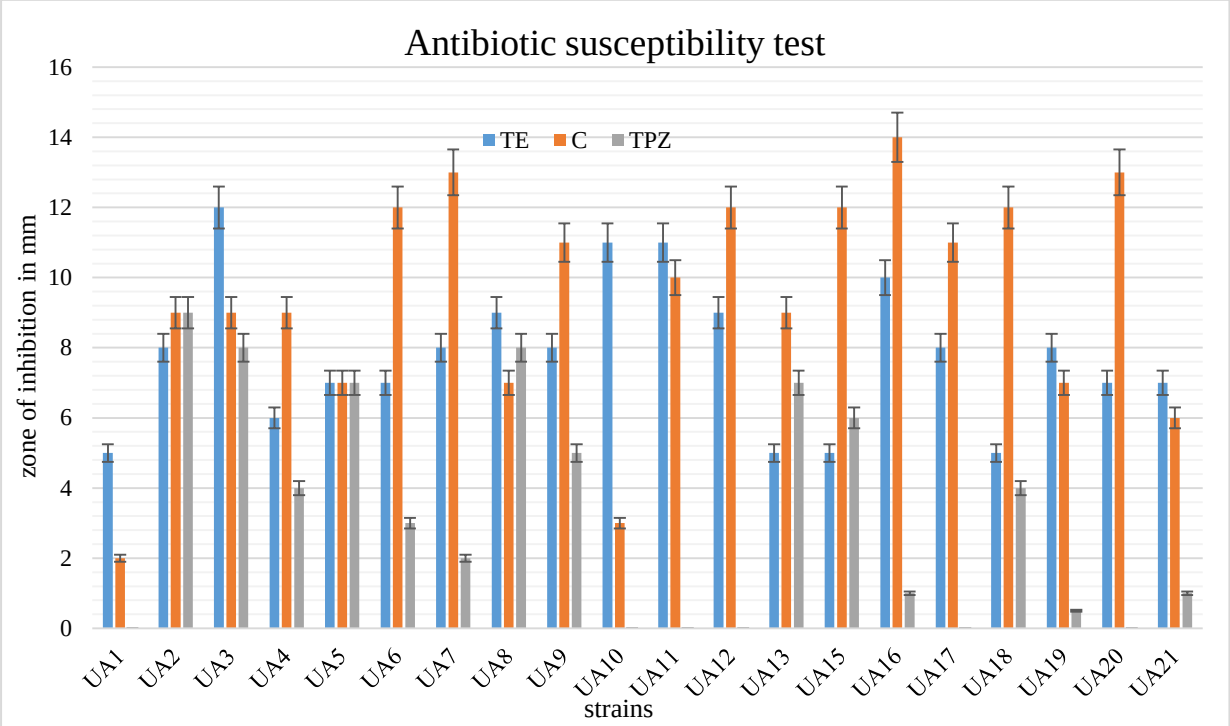

Figure 3: Antibiotic susceptibility profile: measured as **zone of inhibition (mm)** against three antibiotics**: TE (Tetracycline), C (Chloramphenicol), and TPZ (piperacillin).**

Table 3: Heavy metal resistance profile

Isolate	lead	lithium	chromium	nickel	copper	cobalt	mercury
UA1	R	S	R	R	S	S	S
UA2	R	S	R	R	S	S	S
UA3	R	S	R	R	S	S	S
UA4	R	S	R	R	S	S	S
UA5	R	S	R	R	S	S	S
UA6	R	S	R	R	S	S	S
UA7	R	S	R	R	S	S	S
UA8	R	S	R	R	S	S	S
UA9	R	S	R	R	R	S	S
UA10	R	S	R	R	R	S	S
UA11	R	S	R	R	S	S	S
UA12	R	S	R	R	S	S	S
UA13	S	S	S	S	S	S	S
UA15	R	S	R	R	S	S	S
UA16	R	S	R	R	S	S	S
UA17	R	R	R	R	S	S	S
UA18	R	R	R	R	S	S	S
UA19	R	S	R	R	S	S	S
UA20	R	S	R	R	S	S	S

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UA21	R	S	R	R	S	S	S

Effects of pH, Temperature, and Chemical Stress on Bacterial Selenite Reduction

Effect of pH on Selenite Reduction

Isolates UA1-UA21 were grown at pH 5, 7, and 9. At pH 5, there was some growth (optical density ≈ 0.11-0.22). UA2, UA3, UA5, and UA13 exhibited higher growth than the others, while UA6 and UA21 grew less. At pH 7, noteworthy growth was seen (optical density ≈ 0.30-1.45), which means a neutral pH is ideal for most isolates. UA7 and UA10 grew the most at this pH. At pH 9, growth was different for each isolate (optical density ≈ 0.25-0.80). Only UA10 and UA21 grew better in these alkaline conditions.

Figure 4a. Optical Density of bacterial isolates at pH 5

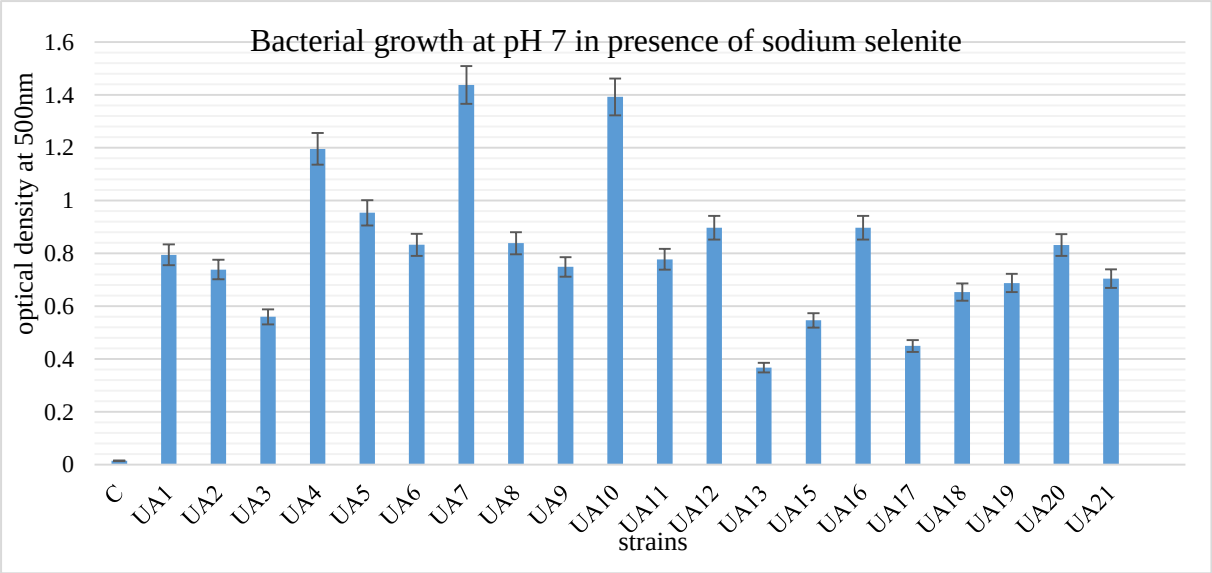

Figure 4b. Optical Density of bacterial isolates at pH 7.

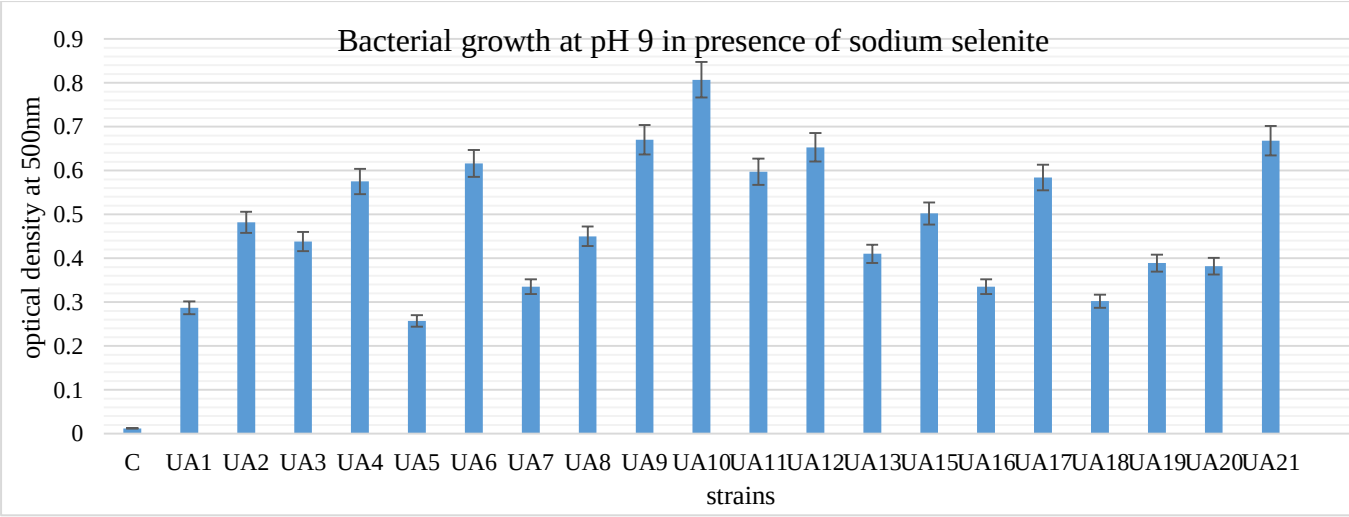

Figure 4c. Optical Density of bacterial isolates at pH 9.

Effect of Temperature on Selenite Reduction

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At room temperature (20-25°C), optical density ranged from 0.37 to 0.89. UA5 showed the highest growth, followed by UA8 and UA3. At 37 °C, higher optical density values (up to 1.438) were recorded, especially for UA7, UA10, and UA4, confirming that this temperature is optimal for most isolates. At 45°C, all strains maintained growth, although the optical density values decreased (0.293 to 0.563). UA17 and UA15 showed relatively good heat resistance.

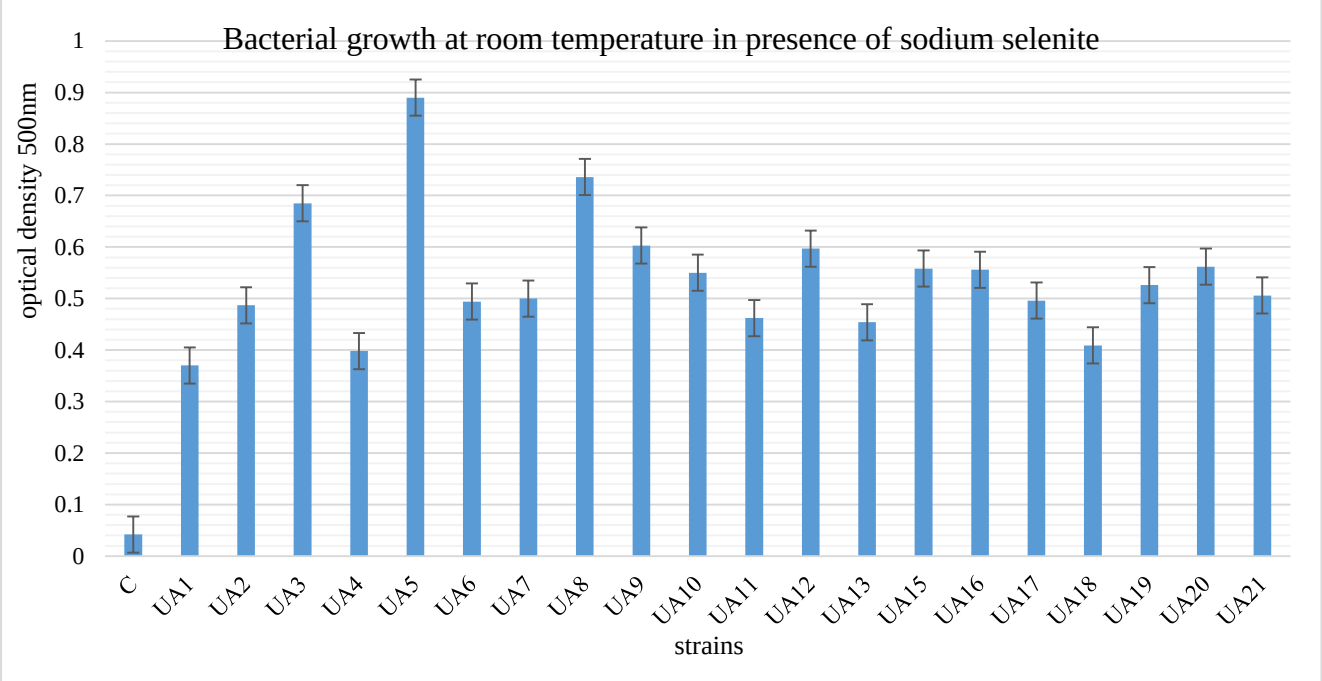

Figure 5a. Optical Density of bacterial isolates after incubation at room temperature.

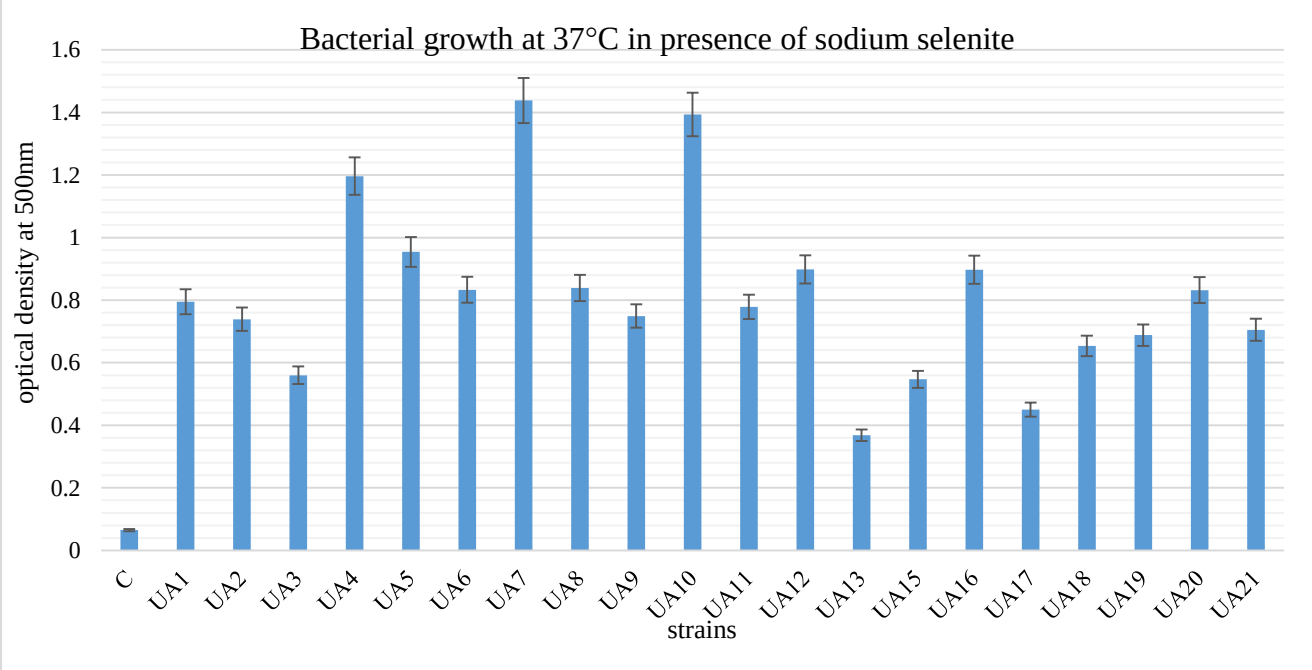

Figure 5b. Optical Density of bacterial isolates after incubation at 37°C.

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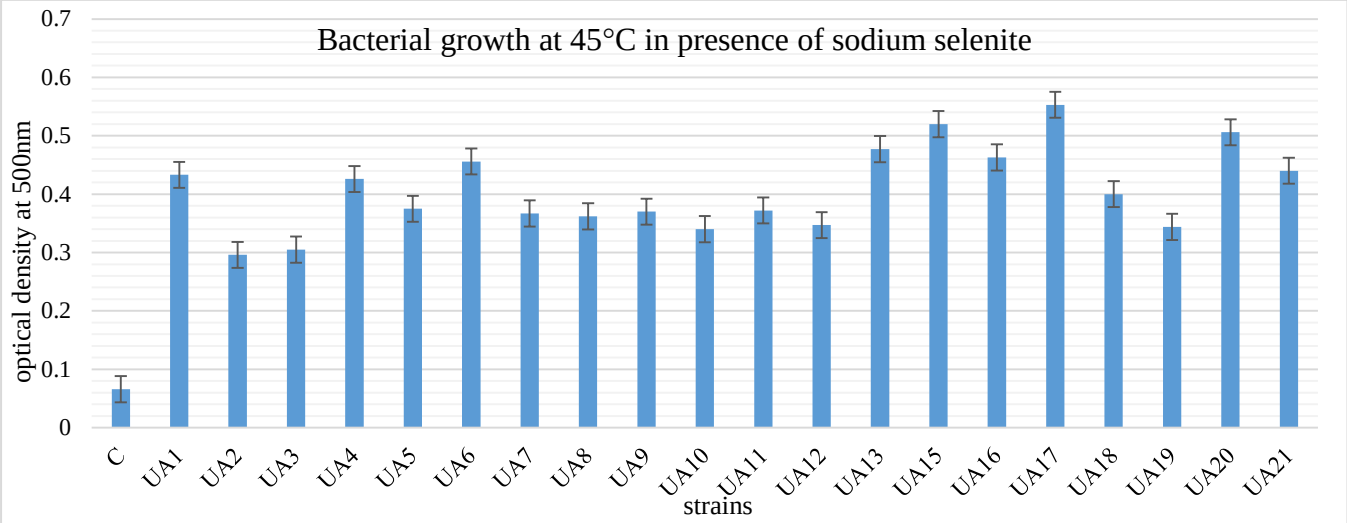

Figure 5c. Optical Density of bacterial isolates after incubation at 45°C

Effect of Incubation Time on Selenite Reduction

After 24 hours, all strains showed measurable growth (optical density 0.036-0.996). The control showed minimal activity, whereas UA13, UA16, and UA12 showed the highest optical density values, indicating early metabolic adaptation. After 48 hours, optical density values increased significantly (from 0.015-1.438), with UA7, UA10, and UA4 showing the highest increase, reflecting increased growth and strain variation. After 72 hours, a growth peak (0.067-2.001) was observed, especially for UA16, UA6, UA13, and UA12 (>1.8), indicating that bacterial growth and metabolic activity were maximal at this time.

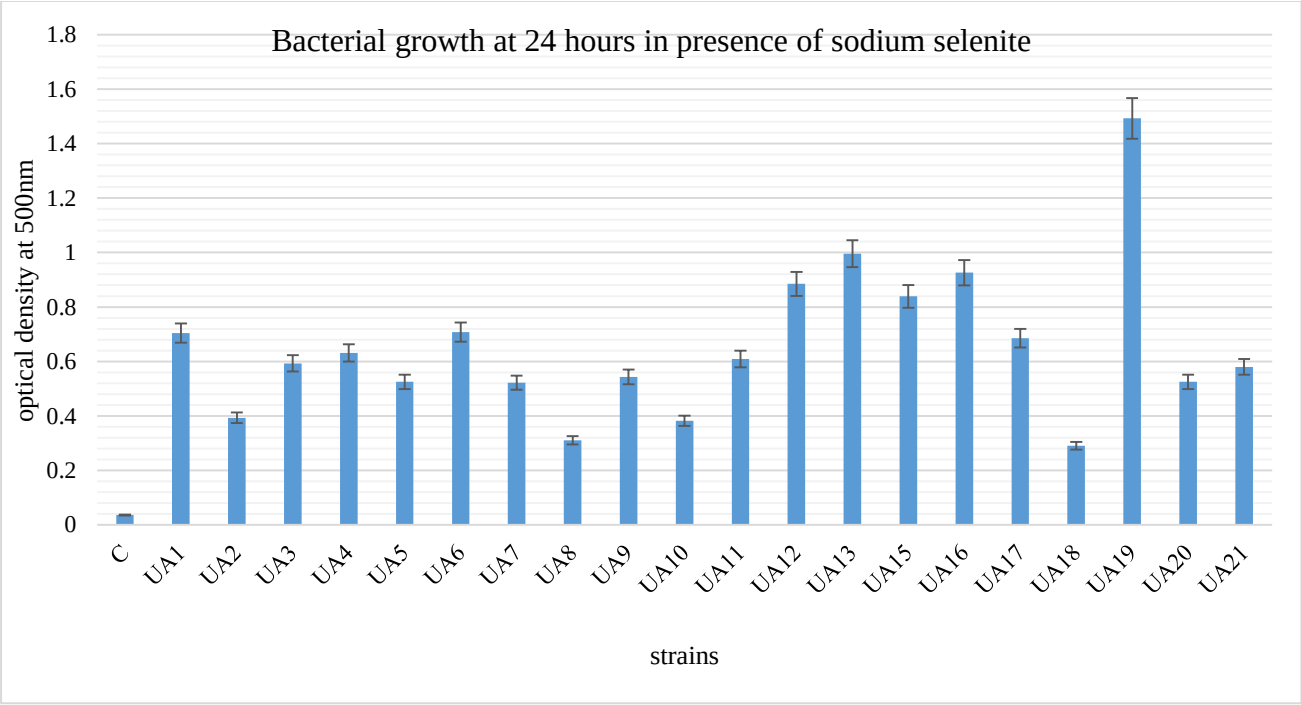

Figure 6a. Optical Density of bacterial isolates after 24 hours of incubation at 37°C

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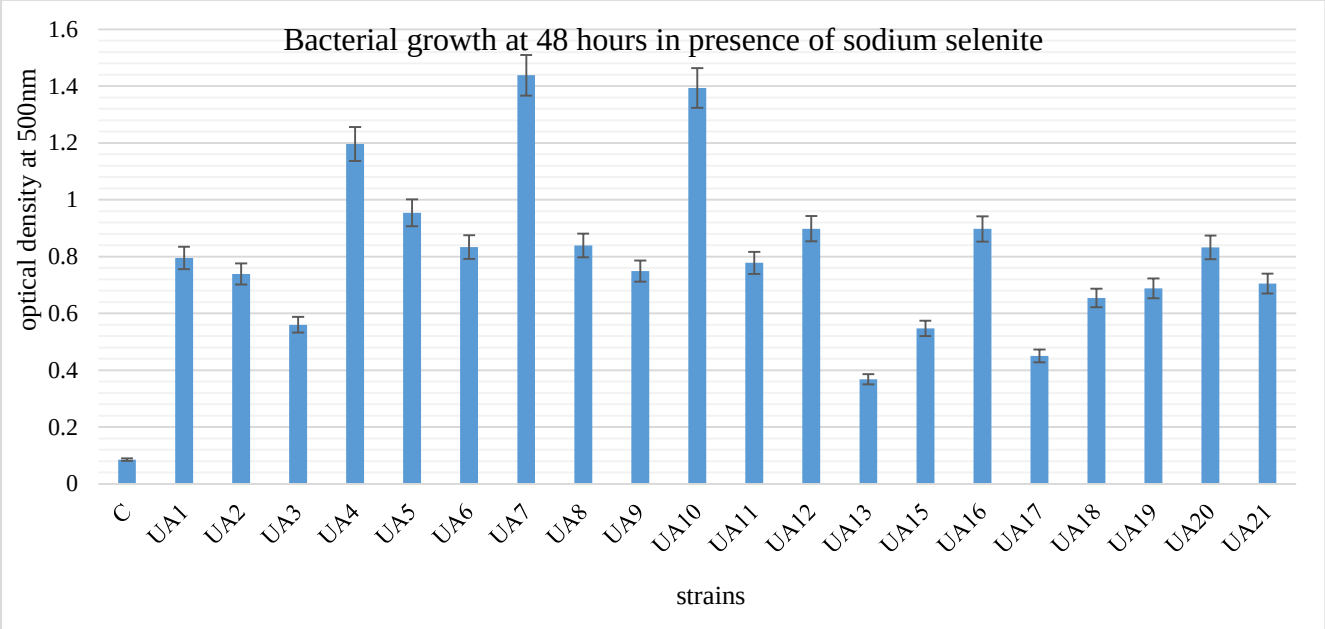

Figure 6b. Optical Density of bacterial isolates after 48 hours of incubation at 37°C

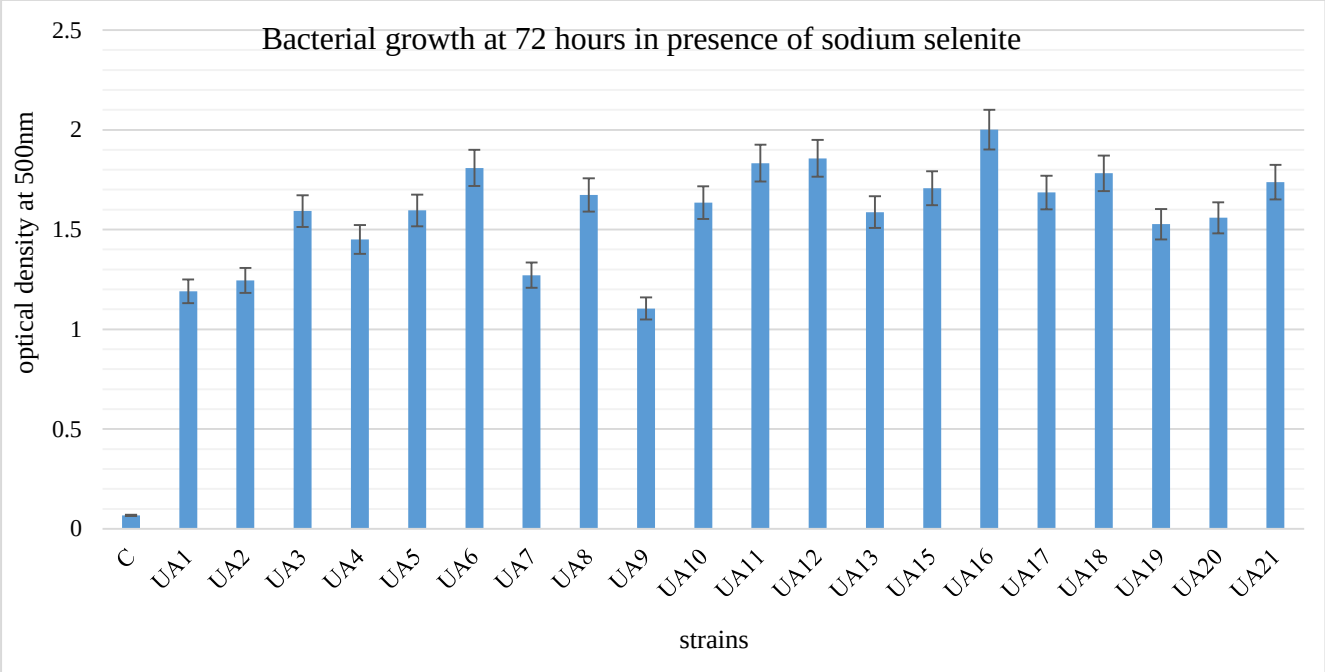

Figure 6c. Optical Density of bacterial isolates after 72 hours of incubation at 37°C

Effect of NaCl Concentration

Bacterial growth as NaCl concentration increases due to salt stress. At 0.5% NaCl**,** most strains, mainly UA2, UA9, and UA17, showed higher growth. At 1.5% NaCl**,** an overall decline in growth was seen, though UA9 and UA10 sustained comparatively higher values. At 2.5% NaCl**,** strains UA3, UA9, and UA15 exhibited strong tolerance as compared to others. The control showed consistently low growth under all conditions.

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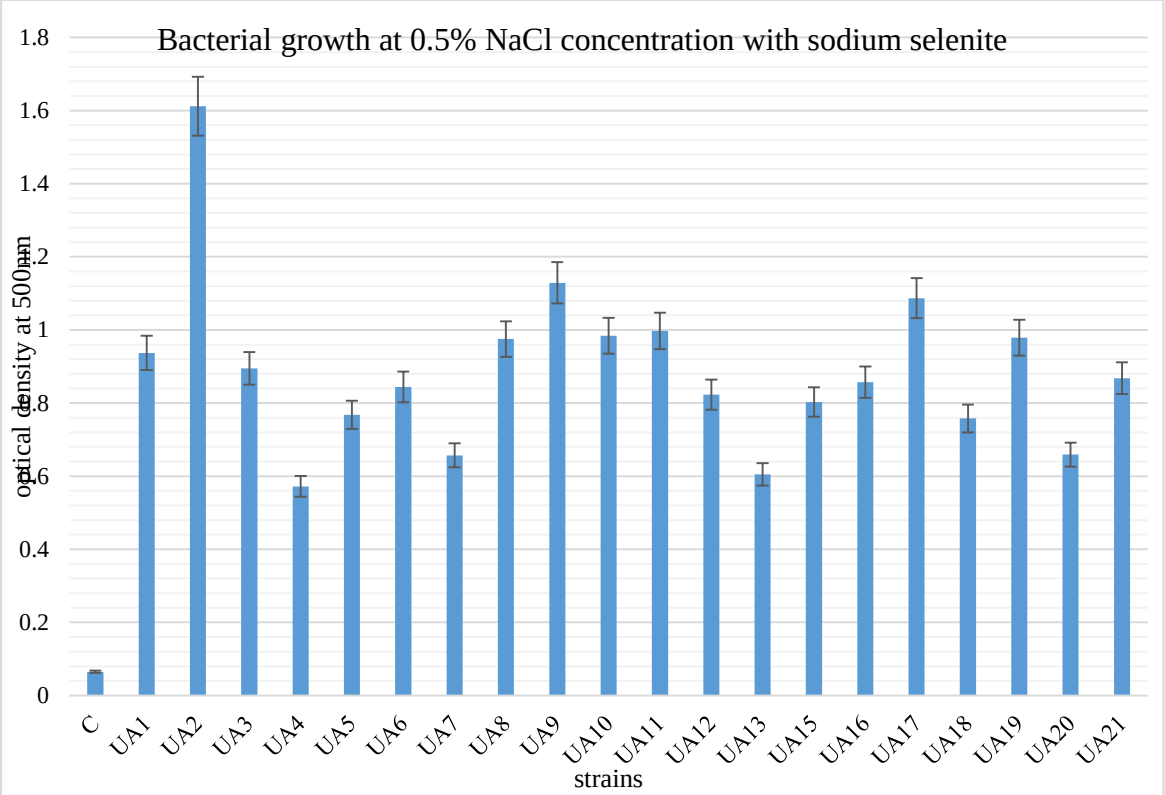

Figure 7a. Optical Density of bacterial isolates supplemented with 0.5% NaCl after incubation at 37°C

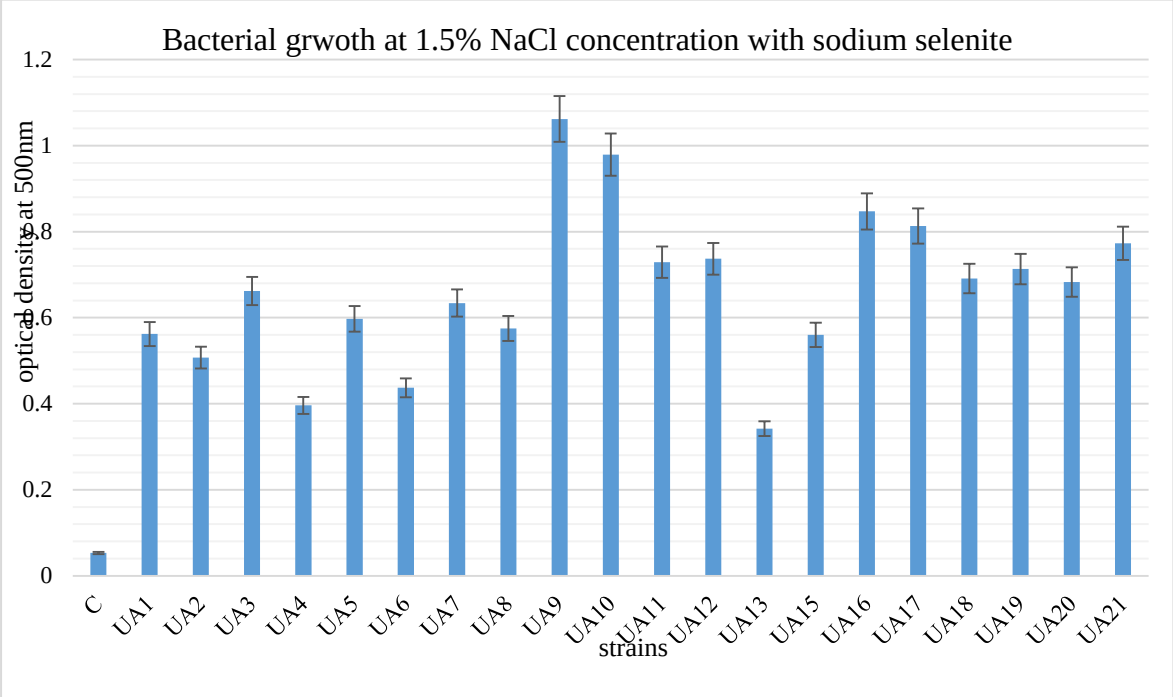

Figure 7b. Optical Density of bacterial isolates supplemented with 1.5% NaCl after incubation at 37°C.

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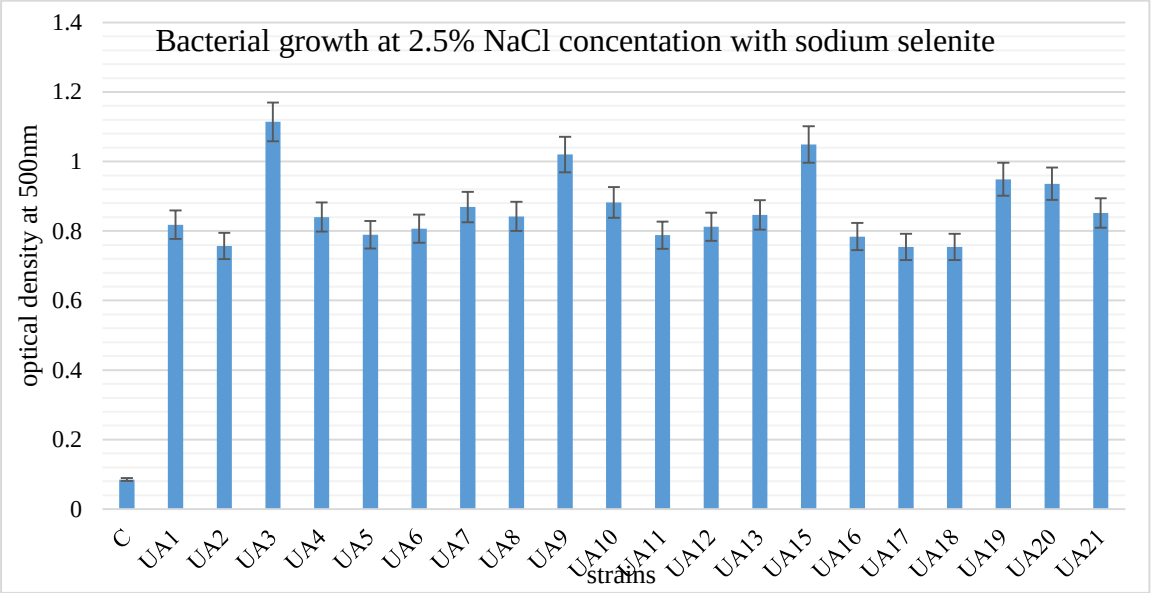

Figure 7c. Optical Density of bacterial isolates supplemented with 2.5% NaCl after incubation at 37 °C

Effect of Sodium Selenite Concentration

We also evaluate growth at 400, 800, and 1200 µg/mL. At a 400 µg/mL concentration of sodium selenite. The optical density was about 0.40 to 1.45, and overall UA7 and UA10 performed better than the rest. At 800 µg/mL, the growth differences became more pronounced (optical density ≈ 0.55-1.60). UA17, UA20, and UA21 continued to grow more than the others. At 1200 µg/mL, the optical density was about 0.35 to 1.50. UA12, UA13, UA16, and UA21 were more resistant, while some isolates did not grow as much.

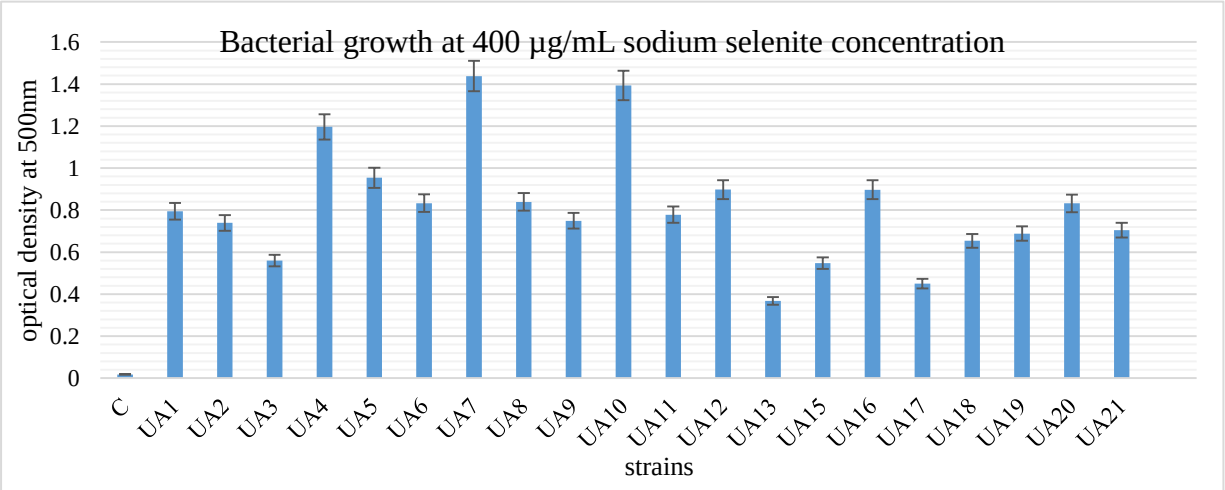

Figure 8a. Optical Density of bacterial isolates supplemented with 400 µg/mL of sodium selenite

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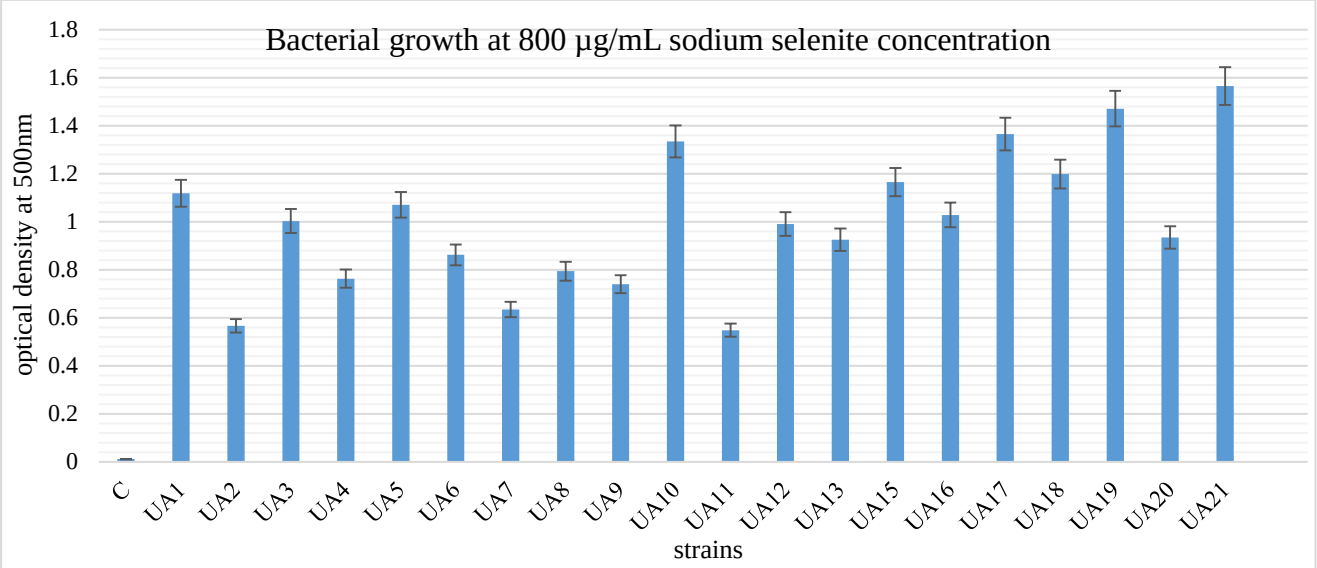

Figure 8b. Optical Density of bacterial isolates supplemented with 800µg/mL of sodium selenite.

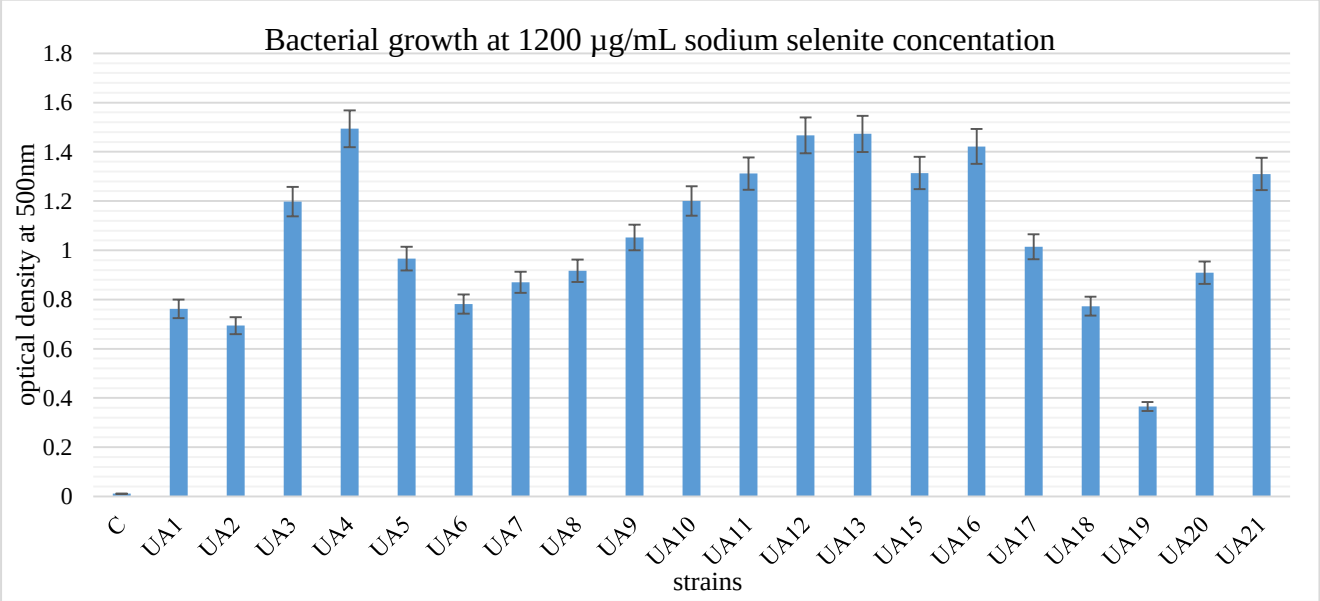

Figure 8c. Optical Density of bacterial isolates supplemented with 1200 µg/mL of sodium selenite.

Discussion

The present study demonstrates the successful isolation and characterization of selenite-reducing bacteria from various environmental samples from Lahore. This indicated that local soils and waters contain metabolically diverse microbial populations capable of transforming selenite. The formation of red elemental selenium by all isolates confirms the active reduction of selenite. This is a widely reported detoxification mechanism in environmental bacteria where soluble and toxic selenite is converted to insoluble elemental selenium [29, 30]. This reduction not only reduces the toxicity of selenite but also maintains its stability in polluted

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environments. Most isolates were also Gram-positive. The endospore-forming strains suggest that many isolates may be derived from stress-tolerant species such as *Bacillus*. Metal tolerance and environmental tolerance are associated with the genus *Bacillus* [33]. Endospore formation provides valuable ecological benefits under harsh environmental conditions. These include heavy metal stress and the oxidizing environment, which is created by selenium oxyanions. The dominance of spore-forming bacteria in selenium- or metal-contaminated areas is supported by their survival strategy, as shown experimentally [33].

Biochemical profiles revealed the frequent presence of catalase, oxidase, starch hydrolysis, and nitrate-reducing activities. Positive catalase and oxidase results indicate active aerobic metabolism and efficient reactive oxygen species detoxification. These mechanisms are important in the reduction of selenite, as redox changes can cause oxidative stress [28, 29]. The observed large-scale nitrate reduction capacity is consistent with previous results. According to their data [28, 31], the reductase system involved in nitrate metabolism may also be involved in the selenite reduction pathway. This is an important discovery. The isolates possess flexible respiratory mechanisms capable of degrading selenite under different environmental conditions, as evidenced by their jointly observed metabolic capacities. Surprisingly, none of the isolates was able to solubilize phosphate. This means that the selenite reduction effect does not correspond to classical plant growth-promoting traits. Nevertheless, most produce indole-3-acetic acid and ammonia. These characteristics influence root growth and nitrogen availability [35, 36]. The coexistence of reduced selenium and certain properties of PGPR supports the possibility that these bacteria can function in selenite-contaminated agricultural soils without significantly compromising plant-microbe interactions [36]. Antibiotic susceptibility testing showed variability. However, universal susceptibility to ceftazidime has been observed. This suggests that although environmental isolates are showing adaptation, they may not yet possess broad clinical resistance determinants. The presence of multimetal resistance in different isolates is noticeable. Most isolates were found to be resistant to lead, chromium, and nickel. Sensitivity to lithium, cobalt, and mercury was more common. Heavy metal tolerance often overlaps with the selenite detoxification mechanism. These include efflux systems, oxidoreductases, and various stress response pathways. In polluted environments, multi-metal resistance is quite common. This may be due to the involvement of the same genetic and physiological tools.

Selenite reduction activity across a range of pH, temperatures, salt levels, and incubation times suggests that these bacteria adapt well to different conditions. That kind of flexibility is important for bioremediation efforts, as environmental parameters can change. [29, 30]. The reduction of environmental mesophiles at mesophilic temperature and neutral pH is consistent with previous studies. [29]

Overall, the results are consistent with international reports on the critical role of environmental bacteria in the cycling of selenium and their potential as agents for bioremediation and sustainable agriculture. [29, 34, 36]. However, the discovery of specific molecular components and their genetic examination will yield more knowledge about their mechanisms.

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Conclusion

The study indicates that environmental samples from Lahore are abundant in selenite-reducing bacteria with distinct morphological, biochemical, and physiological characteristics. These isolates exhibited good reduction of selenite to elemental selenium, heavy-metal tolerance, and selected plant growth characteristics, particularly auxin and ammonia production. Their ability to withstand diverse environmental conditions indicates their ecological potential and use in selenium bioremediation. Although no phosphate solubilization was observed, the reduction in selenium and the presence of other desirable characteristics indicate that these microorganisms can be used in integrated environmental and agricultural applications.

Conflict of Interest

The authors declare no conflict of interest regarding the publication of this study.

Funding Statement

This research did not receive any specific grant.

Ethical Approval

No ethical approval was required as no human participants or animals were involved.

Author Contributions

Alishba Sadia: methodology, experimentation, data analysis, and manuscript writing.
Ubaidullah Naeem: Data collection, laboratory work, and manuscript review.
Dr. Ayesha Siddiqa: Supervision, project administration, and critical revision of the manuscript.

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