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ORIGINAL ARTICLE

Influence of Chemolithotrophic Bacteria on Corrosion Rates of Galvanised and Mild Steel in Sea and Tap Water

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ABSTRACT

Microbiologically influenced corrosion (MIC) represents a complex interplay between microbial communities, their metabolic activities, and the physicochemical characteristics of the surrounding environment. This study investigated the roles of diverse bacterial isolates in the corrosion of mild and galvanised steel exposed to seawater and freshwater systems. Galvanised and mild steel bars were obtained, prepared and suspended in sea, tap, and sterile distilled water for 365 days. Physicochemical parameters and bacterial loads of the water samples were monitored at an interval of 90 days. Bacteria were isolated on Baar's and 9K media. Corrosion rates of the steel samples were estimated across the different water samples. Isolated bacteria were identified by means of conventional biochemical and molecular methods using 16S rRNA gene. The identified bacterial isolates, *Clostridium beijerinckii, Bacillus altitudinis, Alcaligenes faecalis, Stenotrophomonas maltophilia*, *Clostridium diolis* and *Neobacillus niacin*, play mechanistically distinct yet synergistic roles in corrosion processes. Strict anaerobes such as *Clostridium* spp. contribute to MIC via biofilm formation and production of corrosive metabolites, including organic acids and sulphides, which destabilize metal surfaces. Facultative and aerobic organisms such as *Alcaligenes* and *Bacillus* spp. influence corrosion through biosurfactant production, oxygen depletion, and extracellular polymeric substance (EPS) synthesis. The corrosion rate was highest in mild steel, with 19.97 µm/Yr in sea water at day 365. The formation of multispecies biofilms was identified as a critical functional driver of corrosion, enabling metabolic cooperation between aerobic and anaerobic populations. The study accentuates the role of bacteria in supporting an increased rate of steel corrosion.

Keywords: Corrosion rate; Bacteria; Biofilm; Mild steel; Galvanised steel.

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1.0 INTRODUCTION

Metallic corrosion is a menace that has plagued many industries and crippled several industrial processes. Corrosion can cause significant damage to metal structures and components, reducing their strength, functionality and aesthetics (Assad et al., 2025; Zehra et al., 2025). The properties of metals, such as ductility, malleability and conductivity, make them quite essential in many industries and industrial processes (Gu et al., 2019). Metals come in different forms and are utilised for different purposes; they are essential for the development and functioning of modern societies; especially in building and construction industries, machinery and automobiles, fishing and water transportation, etc. (Csavina et al., 2012). Corrosion of metals is the process of gradual deterioration of the metal surface due to chemical or electrochemical reactions with the environment. It can also lead to safety hazards, environmental pollution and economic losses (Singh et al., 2018; Videla, 2003). Two major types of corrosion have been identified, *viz,* uniform and localised corrosion. Uniform corrosion arises when the corrosion occurs over the entire surface of the metal, often due to a change in the position of anodic and cathodic sites formed on the metal surface with relation to time. Conversely, localised corrosion occurs at only certain portions of the metal surface, resulting from the fixed position of both anodic and cathodic sites on the metal surface (Etim et al., 2021). The area under the corrosion deposits is usually devoid of oxygen, and hence serves as the anode, while the areas on the metal where there is no corrosion deposit usually have a high amount of oxygen, and hence serve as the cathode. Crevice corrosion, pitting corrosion and stress corrosion cracking are among the common forms of localised corrosion (Aouniti et al., 2016). Crevice corrosion occurs at crack sites, pitting corrosion creates holes on the metal surface, while stress corrosion occurs when metal is under mechanical stress (Dordevic et al., 2021). Microbiologically influenced corrosion (MIC) of metals is a process that involves the deterioration of metallic materials due to the presence and metabolic activity of microorganisms (Li & Ning, 2019). This type of corrosion can cause significant economic losses and environmental damage, as well as pose a threat to human health and safety (Emerson et al., 2010). MIC can affect various metals, such as iron, steel, copper, aluminium, zinc, and nickel, and can occur in different environments, such as soil, water, oil, and gas pipelines, industrial plants, and marine structures. Biocorrosion Biocorrosion is the deterioration of metals caused by microorganisms, whose metabolic activities produce substances that can take electrons from the metal surface (Obidi et al., 2023). MIC has been a serious challenge in industries for several decades; however, it is often poorly recognised and understood (Machuca, 2019). Bacterial corrosion (biocorrosion) is a complex process influenced by factors such as the type of microorganism, the nature of the metal, environmental conditions, and the presence of other microbes or organic matter. A key mechanism underlying biocorrosion is the formation of biofilms on metal surfaces. These biofilms, composed of single or mixed microbial species, develop when bacteria attach to the metal and secrete extracellular polymeric substances (EPS) (Omar et al., 2021). The biofilm matrix creates a protective microenvironment that enhances microbial survival and activity. Within this structure, bacteria produce corrosive metabolites such as acids, sulphides, and hydrogen, and facilitate processes like cathodic depolarisation. The biofilm also enables

cooperative interactions among microorganisms, allowing them to break down complex compounds more efficiently, which further accelerates metal deterioration (Pantaléon et al., 2014). Most microbes associated with MIC are grouped under chemotrophs due to their dependence on chemical compounds as energy sources. Majority of microbiologically induced corrosion are caused by anaerobic sulphate-reducing bacteria and aerobic iron oxidising bacteria. Iron bacteria have been described to be able to catalyse the oxidation of ferrous ion (Fe^{2+}) to ferric ion (Fe^{3+}), often causing the latter to precipitate ochre-like deposits. Some of the iron bacteria that have been studied include: *Gallionella, Leptothrix* and *Siderocapsa* (Hedrich et al., 2011). According to Dobretsov & Rittschof (2023), the presence of chloride ions can increase the corrosion rate, and the iron-oxidising bacterial activity may result in the formation of ferric chloride, which is highly corrosive and may concentrate under ferric oxide deposits. Consequently, corrosion rates are usually higher in a marine environment than in a freshwater environment. Bacteria associated with metal corrosion include iron-oxidizing species such as *Gallionella ferruginea* and members of the genus *Acidithiobacillus* (formerly *Ferrobacillus*), as well as sulphate-reducing bacteria (SRB) such as *Desulfovibrio* and *Desulfotomaculum*, and sulphur-oxidizing bacteria (SOB) such as *Thiobacillus*. These microorganisms contribute to corrosion through metabolic activities that produce corrosive agents like ferric iron, hydrogen sulphide, and sulphuric acid. Some bacteria that produce acidic metabolites have also been implicated in microbiologically induced corrosion. SRB, under anaerobic conditions, convert sulphate to sulphide, such as H_2S and HS, which enhance pitting (Diao et al., 2023), while SOB such as *Thiobacillus* sp., *Pseudomonas, Acidithiobacillus*, etc. are usually found oxygenated environment; and their metabolic activities lower the pH of their environment to acidic which in turn causes corrosion of metals (Kikuchi et al., 2016). This research studies the variations in the rate of corrosion of submerged steel coupons in the presence and absence of bacteria species.

2.0 MATERIALS AND METHODS

Seawater sample was collected at Lagos lagoon (6.51872ºN, 3.48370ºE), Lagos State, Nigeria, while tap water sample was collected at the Faculty of Science, Federal University Oye-Ekiti, Ekiti State, Nigeria. All water samples were collected into oven-sterilised 2-litres glass bottles; and kept on ice packs. Galvanised and Mild Steel bar samples were fabricated at the Department of Materials and Metallurgical Engineering (6.51835ºN, 3.40027ºE), University of Lagos, Lagos State, Nigeria. All samples were properly packed and immediately transferred to the laboratory for analysis withing 48 h.

2.1 Preparation and incubation of steel samples

The initial weight of the galvanised and mild steel bars was measured using an analytical balance, and the diameter, length and width of the steel samples were also measured with a digital vernier calliper. Salinity of all water samples was measured using refractometry method. At the beginning of the experiment, dissolved oxygen (DO) and pH were measured with dissolved oxygen meter and pH meter respectively. The total dissolved solids (TDS), total suspended solids (TSS) were also determined. Total bacterial loads of the water samples (sea, tap and sterile distilled water) were estimated by plating 1 mL of diluted (10^{-7} dilution factor) water samples on nutrient agar medium (Hi media). Each steel bar was decontaminated by steeping 70% ethanol for 5 minutes and then suspended in 1000 mL each of seawater and tap water. For control experimental setup, 2000 mL of distilled water was sterilised in an autoclave, allowed to cool, and mild and galvanised steel bars were suspended in 1000 mL of sterile distilled water. The steel samples were incubated in tightly covered and labelled plastic buckets. Each experimental setup was done in triplicate, leading to a total of eighteen (18) experimental setups (Napoleão et al., 2018; Hu et al., 2024).

2.2 Isolation of bacterial strains

The total bacterial load of each water sample was analysed by plating 1 mL of the serially diluted water (10^{-2}, 10^{-4} and 10^{-6} dilution factors) sample on Nutrient Agar medium, incubated at 37°C for 24 h, while Baar's medium (2g $MgSO_4$, 1g $CaSO_4$, 1g NH_4Cl, 5g sodium citrate, 400 mL distilled water; 0.5g K_2HPO_4 200 mL distilled water; 1g yeast extract, 1 mL lactic acid, 400 mL distilled water; filter-sterilised 5% $Fe(NH_4)_2(SO_4)_2$) was used to isolate sulfate bacteria. Each media (sea, tap, and control water samples) set up was performed in triplicate and the 9k medium plates were incubated aerobically while Baar's medium plates were incubated anaerobically in anaerobic jar containing gas packs at 37°C for 72 h. After incubation, the total bacterial load was estimated by counting the number of colonies on the petri plates. The bacterial loads were recorded in CFU/ml with respect to the dilution factors used (Ismail et al., 2014; Hu et al., 2024).

2.3 Cultural, morphological and biochemical characterisation of bacterial isolates

The isolated bacterial strains were characterised culturally by observing certain colonial features (such as colour, margin, shape, surface elevation, size and texture) of the bacterial colonies. Morphology of the cells of each isolated bacterial strain was examined under a light microscope after performing the Gram staining procedure. Biochemical tests such as catalase, indole, acid-fast staining, spore staining, sugar fermentation, motility, methyl red test, Vogues Proskauer, starch hydrolysis, haemolysis, oxidase test and biofilm formation test (Congo red method) were carried out to further characterise the isolates (Kalyuzhnaya et al., 2011).

2.4 Determination of physicochemical parameters

Physicochemical parameters measured at 90 days interval include: pH (measured with the aid of calibrated pH meter), dissolved oxygen (measured with digital DO meter), and temperature (measured with mercury thermometer). The total suspended solids which was estimated by filtering 25 mL of the incubating media (tap, sea, and sterile distilled water samples) with Whatman's No 1 filter paper. After filtration, the filter paper was dried in an oven at 105^0C for 1 h, and the dry-weight of the paper and sample was measure on analytical balance. The weight of suspended solids was estimated using equation (1). The total dissolved solids of the incubating media were determined by heating 20 mL of filtered water samples to dryness in a crucible at 105ºC in an oven. After cooling the crucibles in a desiccator, the dry-weight of the crucible and sample was measured on analytical balance. The weight of the residue was estimated using equation (1). The weight and physicochemical parameters were measured at a 90 day interval (Ali & Fulazzaky, 2020).

$$\text{Weight of residue} = \frac{(W1 - W0) \times 1000}{\text{Vol. of sample (ml)}} \text{ g/ml} \quad (1)$$

W_1: weight of crucible and residue/ weight of filter paper and residue, W_0: weight of dry empty crucible/ weight of dry empty filter paper.

2.5 Determination of corrosion rates in steel samples

Change in the weight of both mild and galvanised steel samples incubated in the three (3) water samples was measured at ninety (90) day intervals till the experiment was terminated at 360 days. The rate of corrosion in the steel samples was estimated according to the method of Lv et al. (2019), using the following mathematical expressions:

$$C_{rate} = \frac{\Delta W}{A \, x \, D \, x \, T} \quad \text{(mm/Yr)} \quad (2)$$

Where;

C_{rate}: is the corrosion rate, ΔW: is the weight loss, T: is the duration of exposure (in days), A: is the surface area of the steel materials, D: is the density of the steel sample.

$$\text{Surface Area} = 2(L \, x \, B \, + \, L \, x \, T \, + \, B \, x \, T) \, cm2 \quad (3)$$

$$\text{Volume of steel bar} = L \, x \, B \, x \, T \, (cm3) \quad (4)$$

L: length of steel bar, B: breadth of steel bar, T: thickness of steel bar

$$\text{Density of steel bar} = \frac{m}{vol.} \, (g/cm^3) \quad (5)$$

m: mass of steel bar, vol.: volume of steel bar

2.6 Molecular identification of bacterial isolates

A 16S rRNA gene identification was carried out to confirm the identity of the bacterial isolates. The genomic DNA of bacterial samples were extracted from 200 µl of 24-h bacterial cultures, using Accu prep Genomic DNA extraction kit. Specific segment of the DNA was amplified through polymerase chain reaction (PCR), using 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R 5'-CGGTTACCTTGTTACGACTT-3' as forward and reverse primers respectively. Using a total of 35 cycles, the PCR conditions are: pre-

denaturation: 94°C for 5 min, denaturation: 94°C for 40 s., annealing: 54°C for 40 s., elongation: 72°C for 40 s., and terminal elongation: 72°C for 10 mins. The PCR products were sequenced using ABI 300, and sequenced data of each bacterial isolate were aligned and trimmed on BioEdit sequence alignment editor (version 7.7.1). Trimmed nucleotide sequences were queried on NCBI to obtain the identity of the isolates, and a phylogenetic tree of the isolates was constructed using the neighbour-joining method (bootstrap value: 1000) on MEGA software version 12 (Obidi et al., 2023; Ekene et al., 2024).

3.0 RESULTS AND DISCUSSION

The elemental compositions of the steel samples presented in Table 1 show that Iron (Fe) is the main element in both samples. The percentage quantity of Nickel (Ni) and Chromium (Cr), contributes to improved toughness and corrosion resistance of the steel in galvanised steel samples, was double the quantity in the mild steel samples.

Table **1.** Elemental compositions of mild steel and galvanised steel in weight %

Materials	Chemical Composition wt. %											
	Fe	C	Si	Mn	S	Cr	Cu	Ni	Al	Ti	V	Co
Mild Steel	98.95	0.2621	0.1122	0.3798	<0.009	0.0237	0.0085	0.0166	0.0350	0.0025	0.0093	0.0074
Galvanized Steel	98.53	0.3437	0.0346	0.3093	0.0621	0.0510	0.027	0.0319	0.0571	0.0086	0.0384	<0.001

The salinity of water samples presented in Figure 1 indicates that the seawater samples have the highest salt content (31 PPT) which may affect corrosion rate by influencing the electrochemical corrosion of metals. Physicochemical parameters in Table 2 show that the pH of the seawater decreased significantly from slightly alkaline to acidic. This is probably due to the metabolic byproducts of the microorganisms present in the incubating media, since the control medium (sterile distilled water) showed neutral pH with slight change during the incubation period. The dissolved oxygen in the control water sample was lower than the sea and tap water samples at zero (0) h. This is due to the sterilization of the control water sample, which expels air from the sterilised water; and because the control water sample was not exposed to the air after sterilization to prevent contamination. A steady decrease was observed in the values of dissolved oxygen (DO), which is an indication of increased biological activity. Oxygen is usually required by aerobic microbes for cellular respiration, where it serves as the terminal electron acceptor. The concentrations of DO record in seawater samples were relatively higher than the DO concentrations in tap water samples. A steady increase in the TSS and TDS values across all the water samples recorded. The mild steel has the highest TSS value (7.70 mg/L) and TDS value (4.61 mg/L) in tap water. This increments in the TSS and TDS values are due to the deterioration of the steel bars in the water samples.

According to the data in Figures 2 and 3, a continuous decrease in the total aerobic and anaerobic bacterial counts was observed in both sea and tap water during the incubation period. The aerobic count in the tap water was slightly higher than the anaerobic count, while the anaerobic count was higher than the aerobic count in seawater. However, the seawater sample has higher aerobic and anaerobic counts than tap water. A decrease in both aerobic (4.45 to 2.21 Log CFU/mL) and anaerobic (5.11 to 3.01 Log CFU/mL) counts was observed in seawater samples after 180 days of incubation. This may be due to a decrease in dissolved oxygen, which affected the population of aerobic bacteria. The decrease in the pH value of the water samples may have also contributed to the reduction in bacterial counts in both sea and tap water samples. Bacteria that are unable to survive in acidic conditions were eliminated as the incubating medium became more acidic. The Isolated bacteria were observed to be heterogeneous metabolically (Table 3). While some of the isolates were able to degrade simple and complex carbon sources; including starch, others were able to ferment only simple sugar substrates. It was also noted that 70% of the bacteria isolates tested positive to biofilm formation.

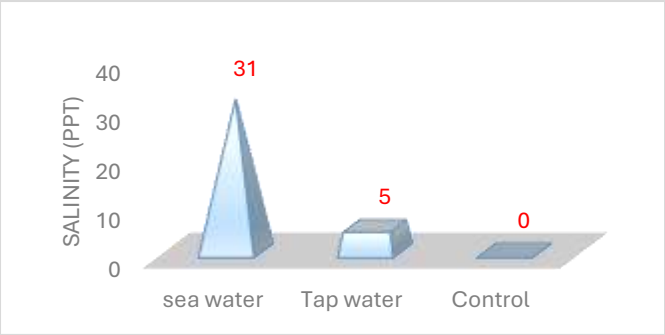

Figure **1.** Salinity level of the water samples

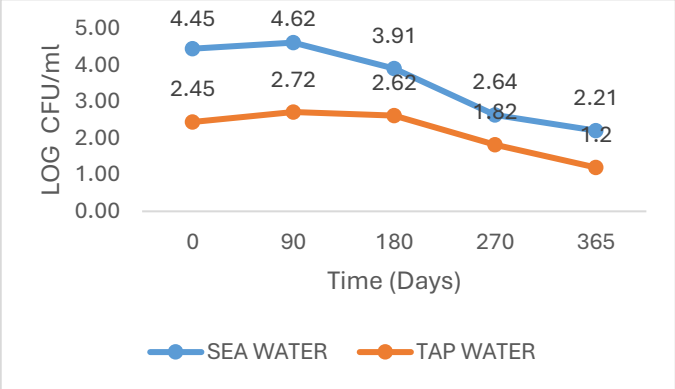

Figure **2.** Total heterotrophic aerobic bacterial count

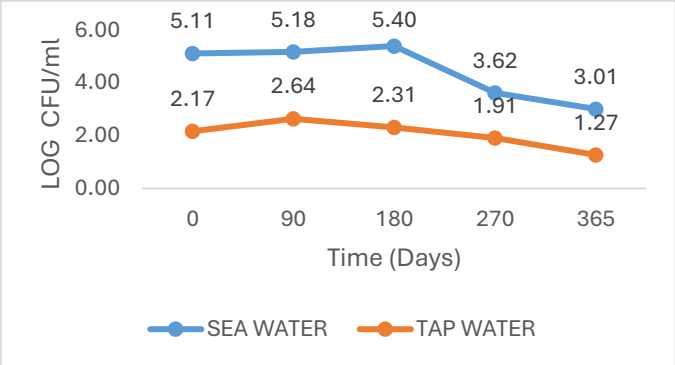

Figure **3.** Total heterotrophic anaerobic bacterial count

Figures 4 present the corrosion rates observed in this study. Minimal corrosion rates were recorded at 90 and 180 days in both galvanized (90 days: 0.98 and 180 days:1.61 µm/Yr) and mild steel (90 days: 2.00 and 180 days:2.36 µm/Yr) samples. The corrosion rates at 270 and 365 days were higher in both galvanised and mild steel samples. The corrosion rate peaked at 17.02 µm/Yr in galvanised steel and 19.97 µm/Yr in mild steel samples. Corrosion rates of galvanised and mild steel samples in seawater were higher than rates observed in tap water and the control. This can be linked to the population of microbes present in both sea and tap water samples, because the microbial population in seawater (4.62 and 5.40 Log CFU/ml for aerobic and anaerobic, respectively) was higher than in tap water (2.72 and 2.64 Log CFU/mL for aerobic and anaerobic, respectively). Corrosion rates in the control samples (sterile distilled water) were lower than rates in tap and seawater; this could be due to the absence of microorganisms in the control samples, since all samples were studied under the same conditions. Statistically, there is negative correlation between total bacterial load and corrosion rate in steel samples (sea water: r = -0.986 ($p = 0.002$), Tap water: r = -0.933 ($p = 0.021$)). This is likely because bacteria capable of scavenging electron from the metals for metabolic processes takes over after the death of less active bacteria. It is also possible that the build-up of microbial metabolic products such as acids, hydrogen sulphides, lowers the pH of the water samples, causing the death of certain bacteria, while at the same time increasing metal deterioration.

The seawater samples (pH 5.2) were observed to have slightly lower pH than tap water samples (pH 6.4); this may have also contributed to the higher corrosion rates in seawater. It was also noted that the corrosion rates in mild steel samples (Figure 4) were slightly higher than in galvanised steel. This may have been due to the quantity of the elemental compositions of both steel samples, because certain elements, such as nickel and chromium, which were added to increase toughness and corrosion resistance, are relatively higher in galvanised steel samples. Generally, data from this study showed that corrosion rates in mild steel is higher than galvanised steel (Plate 1). A two-way analysis of variance (ANOVA) between the metal type and environment (water samples) shows that there is significant difference in the corrosion rates observed in the three water samples ($F = 5.84$, $p = 0.005$).

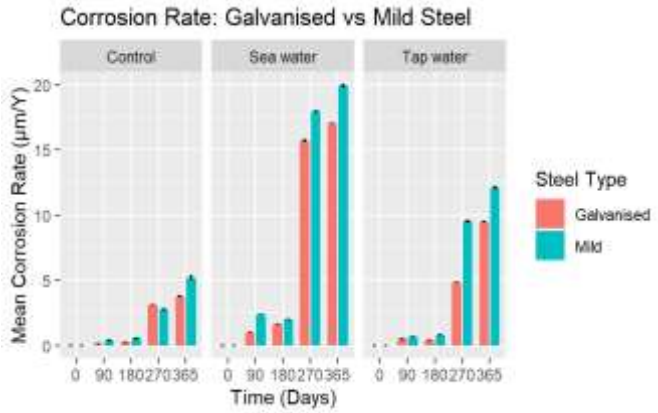

Figure **4.** Corrosion rates of galvanised and mild steel in different water samples

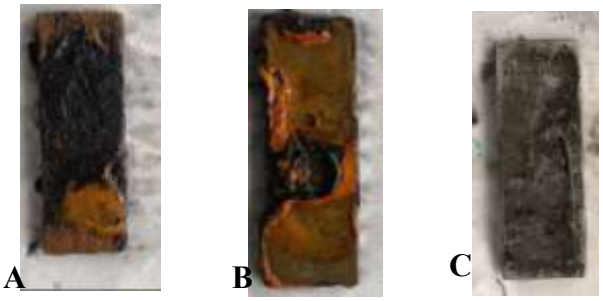

*Plate **1.** Corroded steel samples incubated for 365 days in sea water, tap water and distilled water samples respectively A: mild steel, B: galvanised steel, C: galvanised steel.*

According to the data from this study, the presence of microbes significantly increased the corrosion rates of the steel samples. Ten (10) bacterial isolates were identified molecularly in this study (Table 4). These bacterial isolates have varied genera as well as oxygen requirements. Strict anaerobes such as *Clostridium* sp. and obligate aerobes such as *Alcaligenes, Bacillus, Neobacillus* and *Stenotrophomonas* were isolated in this study.

The evolutionary tree of bacterial isolates represented in Figure 5 shows the level of genetic relatedness among these bacteria. Isolate SGS forms a monophyletic group with *Bacillus vallismorti*, which is in contradiction with the BLAST result that identified this microbe as *Calidifontibacillus erzurumensis*. Isolates SMS, SMS1N and TMS13B are grouped in the same clade with *Clostridium* as suggested by the BLAST analysis. Isolate SGS13, with a branch length of 0.28, although identified as *Hespellia* sp. with a branch length of 0.09, is evolutionarily divergent. This is supported by the BLAST result in Table 4, although *Hespellia* was the closest related microbe, the query cover and percentage identity are low, coupled with a high error value. This strain is suspected to be an unidentified bacterium. Isolate TGS1 appeared to be the most genetically divergent of all the bacteria isolated in this study, with a branch length of 0.56. The tree shows a common ancestor of isolate TGS1 and all the organisms included in the tree from which all other lineages have diverged.

Table **2:** The physicochemical parameters of the water samples during the period of incubation

Water Sample	**Time (Day)**	**pH**		**Temp. (^{0}C)**		**DO (mg/L)**		**TSS (mg/L)**		**TDS (mg/L)**	
		Galvan	Mild	Galvan	Mild	Galvan	Mild	Galvan	Mild	Galvan	Mild
Tap	0	7.1 ± 0.1	7.1 ± 0.1	27 ± 00	27 ± 00	5.2 ± 0.2	5.2 ± 0.1	0.06 ± 0.01	0.06 ± 0.01	0.19 ± 0.01	0.14 ± 0.03
Sea		7.9 ± 0.2	7.8 ± 0.1	27 ± 00	27 ± 01	5.6 ± 0.1	5.6 ± 0.1	0.02 ± 0.01	0.02 ± 0.01	0.08 ± 0.01	0.08 ± 0.01
Control		6.8 ± 0.0	6.8 ± 0.1	27 ± 00	27 ± 00	4.0 ± 0.1	4.0 ± 0.0	0.01 ± 0.02	0.01± 0.02	0.05 ± 0.01	0.05 ± 0.01
Tap	90	7.5 ± 0.1	7.5 ± 0.1	30 ± 00	30 ± 00	4.8 ± 0.1	4.7 ± 0.1	0.07 ± 0.01	0.08 ± 0.01	0.63 ± 0.00	0.66 ± 0.01
Sea		6.7 ± 0.2	7.6 ± 0.2	30 ± 00	30 ± 00	5.3 ± 0.1	5.1 ± 0.1	0.03 ± 0.01	0.86 ± 0.01	0.17 ± 0.01	0.43 ± 0.02
Control		7.1 ± 0.1	7.2 ± 0.1	30 ± 01	30 ± 00	3.8 ± 0.1	3.9 ± 0.1	0.03 ± 0.01	0.04 ± 0.01	0.06 ± 0.01	0.10 ± 0.01
Tap	180	6.9 ± 0.1	7.2 ± 0.1	28 ± 02	28 ± 01	4.4 ± 0.1	4.5 ± 0.3	0.23 ± 0.00	0.11 ± 0.01	0.86 ± 0.01	0.76 ± 0.01
Sea		6.5 ± 0.1	6.9 ± 0.0	28 ± 01	28 ± 00	4.7 ± 0.3	4.9 ± 0.1	0.10 ± 0.01	0.93 ± 0.01	0.21 ± 0.03	0.46± 0.01
Control		7.2 ± 0.1	7.0 ± 0.1	28 ± 00	28 ± 01	3.9 ± 0.2	3.8 ± 0.1	0.06 ± 0.01	0.07 ± 0.02	0.08 ± 0.02	0.12± 0.01
Tap	270	5.6 ± 0.1	5.7 ± 0.3	26 ± 01	26 ± 00	3.8 ± 0.1	3.8 ± 0.1	2.84 ± 0.01	7.52 ± 0.01	0.98 ± 0.01	1.61 ± 0.00
Sea		5.3 ± 0.0	5.1 ± 0.1	26 ± 01	26 ± 00	3.8 ± 0.1	3.9 ± 0.1	1.84 ± 0.01	5.52 ± 0.00	0.27 ± 0.01	0.64 ± 0.01
Control		6.8 ± 0.1	6.9 ± 0.2	26 ± 00	26 ± 01	3.8 ± 0.1	3.5 ± 0.1	0.08 ± 0.01	0.22 ± 0.01	0.19 ± 0.01	0.49 ± 0.02
Tap	365	5.7 ± 0.1	6.4 ± 0.1	27 ± 00	27 ± 02	3.5 ± 0.2	3.5 ± 0.1	5.12 ± 0.00	7.70 ± 0.01	4.28 ± 0.01	4.61 ± 0.01
Sea		5.2 ± 0.3	5.2 ± 0.2	27 ± 00	27 ± 01	3.6 ± 0.1	3.6 ± 0.1	2.37 ± 0.02	6.38 ± 0.00	1.17 ± 0.01	4.16 ± 0.01
Control		6.8 ± 0.1	6.7 ± 0.0	27 ± 01	27 ± 01	3.5 ± 0.1	3.5 ± 0.1	0.25 ± 0.00	0.33 ± 0.00	0.40 ± 0.02	0.66 ± 0.02

Table **3.** Biochemical characteristics of bacterial isolates

Isolate ID	Catalase	Motility	Indole	Starch Hydrolysis	Mannitol	Xylose	Fructose	Lactose	Oxidase	MR	VP	Haemolysis	Acid fast	Spore	Biofilm test	Preliminary identification
TGS1	+	+	-	-	+	+	+	+	-	-	-	β	-	+	+	*Pseudomonas* sp.
SMS1N	+	-	-	-	-	-	-	-	+	-	-	γ	+	+	+	*Clostridium* sp.
TMS15	+	-	+	+	+	+	+	+	+	-	-	γ	+	+	+	*Bacillus* sp.
SGS13	+	-	-	-	+	+	+	+	+	-	-	γ	+	+	-	*Bacillus* sp.
TMS13B	+	-	-	-	+	+	+	+	-	-	-	β	-	+	+	*Clostridium* sp.
SMS13	+	-	-	+	+	+	+	+	+	-	-	β	+	+	+	*Aeromonas* sp.
TGS1B	+	-	+	-	+	-	+	-	+	-	-	α	-	-	-	*Bacillus* sp.
SMS1A	-	-	-	-	-	-	+	-	+	-	+	β	-	-	+	*Clostridium* sp.
TMS1B	+	-	+	-	-	+	+	-	+	-	-	α	-	-	+	*Corynebacterium* sp.
SGS1A	+	-	+	+	-	+	+	-	-	-	-	β	-	-	-	*Bacillus* sp.

Table **4.** Molecular identity of bacterial isolates

Isolate ID	Isolate identity	Percent. similarity (%)	E-Value	Query cover (%)	Ascension number
TGS1	*Stenotrophomonas maltophilia*	88.70	0.0	99	NR_041577.1
SMS1N	*Clostridium beijerinckii*	93.48	0.0	100	NR_113388.1
TMS15	*Alcaligenes faecalis*	98.64	0.0	100	NR_113606.1
SGS1A	*Calidifontibacillus erzurumensis*	95.13	0.0	99	NR_180255.1
TGS1B	*Priestia flexa*	99.34	0.0	100	NR_113800.1
SGS13	*Hespellia* sp.	71.02	$2e^{-12}$	68	KC854374.1
SMS1A	*Clostridium diolis*	92.06	0.0	100	NR_025542.1
TMS1B	*Bacillus altitudinis*	99.74	0.0	100	NR_042337.1
TMS13B	*Clostridium beijerinckii*	100	0.0	100	NR_029230.1
SMS13	*Neobacillus niacini*	98.81	0.0	100	NR_024695.1

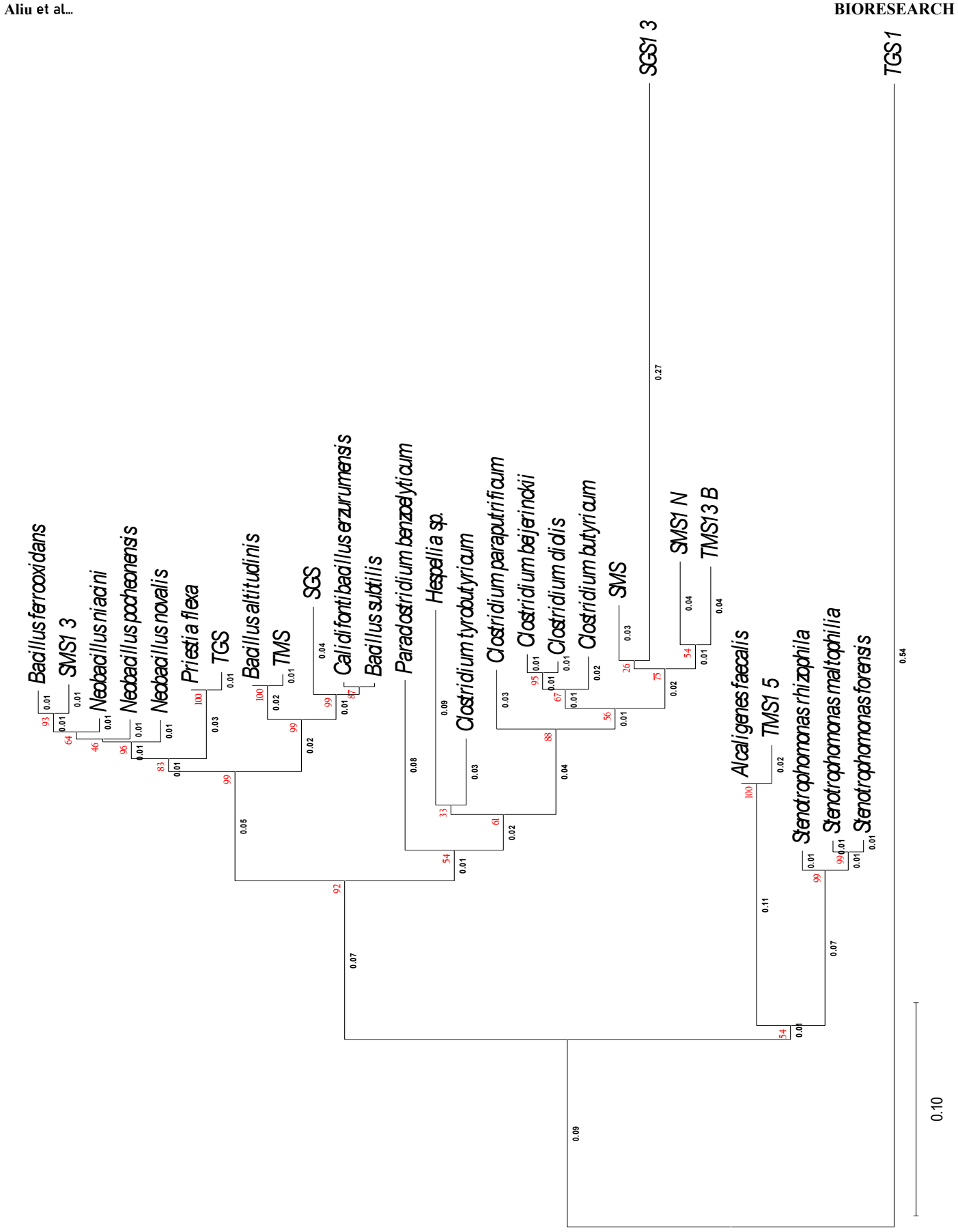

Figure **5.** Evolutionary relatedness of identified bacterial isolates

3.1 Elemental compositions of metal samples

Elemental composition of metals (e.g. steel) plays a pivotal role in determining their susceptibility to microbially influenced corrosion. Heterogeneous microstructures sometimes occur on metal surfaces due to elemental compositions of metals; these microstructures attract and permit microorganisms to establish microbial growth film, which causes metal degradation. According to Al-Eshaikh & Kadachi (2011), the elemental compositions of metal alloys play a decisive role in determining the properties (such as toughness, corrosion resistance, hardness, temperature resistance, etc.) of metal alloys. Studies have shown that the corrosion resistance of any metal alloy is dependent on the addition of several elements, mainly chromium, nickel, copper, molybdenum, silicon and nitrogen (Liu et al., 2022). The corrosion resistance of any metal is dependent not just on the elemental compositions but also on the microstructure that results from the interactions of these elements.

3.2 Physicochemical properties

According to Qian et al. (2021), chloride build-up and higher microbial count in the seawater sample can contribute to a decrease in dissolved oxygen, which in turn supports an increased corrosion rate. Since the experimental setup was conducted at room temperature, there was no significant difference in the temperature values recorded throughout the experiment. Several studies have shown that some physicochemical parameters are closely associated with metal corrosion. According to Tanupabrungsun et al. (2013), acidic pH contributes immensely to the corrosion rate in steel. In a study conducted by Xu et al. (2023), it was also established that lower pH favours a higher corrosion rate, which supports the findings in this current study. Lv et al. (2019), stated that the rate of corrosion in reinforcing steel is inversely proportional to the pH values of the incubating medium. Lower pH increases the cathodic reaction and prevents the formation of protective metal oxides, which cause accelerated corrosion of metals (Jung et al., 2011). In this study, the concentration of dissolved oxygen decreased across the incubation period. This may be linked to the metabolic activities of aerobic microbes present in sea and tap water samples. Dissolved oxygen gradient can be created by the activities of aerobes, especially iron-oxidising bacteria, which oxidise ferrous iron to ferric deposits; this oxygen gradient can lead to localised corrosion (Telegdi et al., 2017).

3.3 Bacterial influence on corrosion rate of metals

The metabolic activities of microorganisms can significantly increase corrosion rates of metals. The presence of certain groups of microorganisms, such as sulphate-reducing bacteria (e.g. Desulphobacter) and iron-oxidising bacteria (e.g. Gallionella), increases corrosion rates by a great margin. The cohabitation of both aerobic and anaerobic bacteria suggests a profound relationship among these bacterial species, probably through the formation of biofilms (Adebesin et al., 2026; Imefon et al., 2024). Aerobic bacteria utilise the oxygen within the environment, and this removal of oxygen provides an anoxic micro-environment for the strict anaerobes (Little & Lee, 2015). Bacteria of the genus *Clostridium* are established biofilm-forming microbes which attach themselves to surfaces with the help of extracellular polymeric substance (EPS). The EPS secreted during biofilm formation shields the bacteria from external stress; thereby enhancing survival of the bacteria, which in turn contributes to the deterioration of the steel samples (Pantaléon et al., 2014). A study by Aslan et al. (2024), who reported a corrosion rate of 0.05 mm/Yr, supported that the formation of biofilms on metal surfaces contributes to faster metal deterioration. According to Wang et al. (2024), *Alcaligenes* sp. produce certain antimicrobial substances such as proteoglycans and biosurfactants, which reduce microbial loads and, at the same time, form protective films on metal surfaces. Sulphides, which are metabolic products of sulphate-reducing bacteria (SRB), can be converted to less corrosive compounds (such as thiosulphate and sulphate) by the metabolites produced by *Alcaligenes*. However, Shi et al. (2023) confirmed that *Alcaligenes* sp. produce organic acids, which cause accelerated corrosion. These organic acids (such as short-chain fatty acids) can significantly lower the pH of the incubating medium, which leads to metal oxidation. The biofilm formed by *Alcaligenes* causes an oxygen gradient within the biofilm, which can lead to a reduction of cathodic protection. *Bacillus altitudinis* and *Neobacillus niacin* have both been implicated in corrosion of metals. According to a study carried out by Dong et al. (2022), *B. altitudinis* was found to cause corrosion of stainless steel through the formation of biofilm on metal surfaces.

The metabolic products, such as polyglutamic acid, polyaspartic acids, cyclic peptides and polysaccharides produced by *Bacillus* species, including *Neobacillus niacini,* cause serious pitting in metals (Wang et al., 2020; Babar et al., 2021). Based on the data published by Stancu (2022), *Stenotrophomonas maltophilia,* which was also isolated in this study, was linked to corrosion of carbon steel. Like many other sulphate-reducing bacteria, *S. maltophilia* is a biofilm-forming bacterium that secretes metabolic by-products which cause degradation of metals. Although *Calidifontibacillus erzurumensis, Priestia flexa* and *Hespellia* sp. have not been established as causative agents of metal corrosion by previous studies, their ability to cohabit with sulphate-reducing bacteria could suggest their participation in metal corrosion (Soto-Varela et al., 2024). In addition, *Hespellia* sp., though not a biofilm-former, produces acidic metabolites such as formic, acetic, lactic, and propionic acids, which can aid metal degradation (Whitehead et al., 2015).

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Conflict of interest

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Authors contributions

AKT: conceptualisation, methodology, writing- original draft, OOF: writing- review & editing, material acquisition, AHA: supervision, NCI: writing- review & editing, AAE: investigation, data curation, OOP: writing- review and HSO: investigation, data curation.

Conclusion

Corrosion of metals is a serious concern that can hinder many industrial processes, and water samples that contain microbial consortia showed higher corrosion rates. Marine habitats, due to the presence of high salt content in addition to the presence of microorganisms, produce a higher corrosion rate than freshwater. Most bacteria involved in metal corrosion are biofilm formers; hence, inhibition of biofilm formation will help in the control and prevention of metal corrosion.

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