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Molecular Characterization of Iron-Related Bacteria in Ground Water in Yenagoa, Bayelsa State, Nigeria

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ABSTRACT: Fe (III) compounds which are formed from the oxidation of Fe(II) by iron bacteria precipitates, and forms iron oxides - an extensive orange mats of organic material, which is often found present in the ground water, evident by the rust stain colour seen on almost all the water storage tanks in Yenagoa, Bayelsa State, Nigeria. Hence, this study characterized iron bacteria diversity in groundwater, which is a major source of drinking water in the state. The aim of this study was to isolate, characterize and ascertain the chlorine tolerance level of iron-related bacteria in groundwater in Yenagoa. The bacteria isolates were identified using standard bacteriological methods. The genomic DNA extracted from the pure cultures of bacteria was amplified using 16S rRNA universal primer sets. The in-vitro chlorine tolerance test of the isolates was carried out at varying concentrations of 1ppm-1000ppm. Genotypic analysis of the eight suspected iron related bacteria species revealed a close relatedness to *Myroides odoratimimus* Q11, *Lysinibacillus xylanilyticus* EB-229, *Pseudomonas xiamenensis* B13, *Shewanella chilikensis* B0B2, *Janibacter indicus* YFY001, *Bacillus velezensis, Aeromonas hydrophila subsp. hydrophila* JCM3989, *and Staphylococcus sciuri* SNUDS-18. All eight species of iron related-bacteria identified showed resistance at varying degrees to Chlorine. *Pseudomonas xiamenensis* was observed to have the highest resistance while *Aeromonas hydrophila* had the least resistance. Susceptibility of the isolated strains was recorded at a chlorine concentration of 5000ppm. Our findings revealed that iron- related bacteria populate groundwater in Yenagoa.

Keywords: Chlorine tolerance, Iron-bacteria, Phylogenetic analysis, Ground water

Introduction

With a global drinking water crisis on the horizon and lack of the provision of municipal water supply by government, the Niger Delta region of Southern Nigeria immensely relies on groundwater from shallow boreholes as the primary source of drinking water. The challenge of providing water is met by boreholes that tap groundwater. Boreholes are deep wells of about 100 m mechanically-drilled with electric submerged pumps to tap groundwater reservoirs (Oyem *et al.*, 2014). Although, groundwater is traditionally regarded as being of good natural quality mostly from its geological environment (Szewzyk *et al.*, 2000; Chapelle, 2001) this does not imply that natural groundwater is always considered as portable water (MacDonald and Calow, 2009).

In as much as groundwater has been considered as a good source of drinking water (Younger, 2007), studies show that groundwater is a home for a vast array of microbial communities, and the presence of these microorganisms can alter or influence the quality of groundwater (Bruce Rauner, 2010). Amongst the important members of microbes found in groundwater ecosystem are the iron related bacteria, which are morphologically and phylogenetically heterogeneous prokaryotes. They are generally known for their ability to convert ferrous iron to ferric iron (Stuart, 2015). Iron related bacteria as a group of aerobic bacteria, appears to utilize the oxidation of ferrous and/or manganous ions as a critical component in their metabolic functioning. Iron related bacteria include both iron oxidizing and iron reducing bacteria and these bacteria function under different reduction-oxidation (redox) conditions (Lovely *et al*., 2004).

In addition to altering the groundwater chemistry by the iron related bacteria, they also have significant impact on man-made water systems. The formation of iron-oxide by these microbes can clog pipes, increase steel corrosion, and result in a variety of negative impacts on the industries (Stuart, 2015).
How does iron get to the groundwater? In the lithosphere, iron is the second most abundant metal and the most abundant element by weight. Since the earth's underground rock formations contain about 5% iron, it is not unusual to detect iron in abundance. As surface waters seep down into the aquifer, iron is washed into groundwater systems, hence their abundance, however, a limit of 0-0.3 mg/L of iron is acceptable. Ferrous iron is relatively stable in anoxic environments but it is having a great tendency to undergo spontaneous chemical oxidation by molecular oxygen and the rate at which this occurs depends on temperature, and on the concentration of protons (hydronium ions), dissolved oxygen and ferrous iron.
Akpokodje (1986) reported that the Niger Delta is basically an alluvial plain and that groundwater flows from North to South in the region. The aquifer is directly recharged through the infiltration of rain water. In the Niger Delta, the water table in many areas is close to the surface though subject to seasonal variations. The water table is about 3–4 m in the dry season but rises considerably in the rainy season. Groundwater is the main source of drinking water for over 80% of the populace in the study area.
The strategic significance of groundwater in Yenagoa and the threats posed by the excessive Fe concentration punctuate the presence and activities of iron bacteria in groundwater ecosystem. The isolation of iron bacteria and ascertaining the degree of chlorine tolerance level of iron bacteria were the main objectives of this study.

Materials and methods

Description of the study area: Yenagoa is the capital city of Bayelsa State and is within the Southern Nigeria sedimentary basin (Okiongbo, *et al*., 2019). Yenagoa lies on longitudes 006° 10′ 3.07″ and 00 6° 25′ 10.53″ East of the prime meridian and latitudes 04° 51′ 39.73″ and 05°.2′ 25.53″ North of the equator. The ground surface of Yenagoa is relatively flat and as such it slopes seawards (Okiongbo, *et al*., 2019). The study area has a tropical rain forest climate characterized by rainy season and dry season. The rainy season commences from April to October with a brief dry period in August. The dry season lasts between November and March. The mean annual rainfall is about 4500 mm and about 85% of the mean annual rain falls during the wet season. The temperature varies between 25 to 32 °C. Fishing and farming are the main occupation of the people. The study area lies within the fresh water swamps, back swamps, deltaic plain, alluvium and meander belt geomorphic unit of the Niger Delta (Akpokodje, 1986).
Sample collection: Groundwater samples were collected at 5 different borehole points. The water was allowed to flow for over three minutes before collecting water samples aseptically, in a sterile labelled bottle. The samples were stored in cooler containing ice and transported to the laboratory within 4 hours.
Sample analysis: Iron bacteria medium was used for culturing the iron related bacteria present in the samples. The plating of the groundwater samples was done in triplicates using pour plate method. Thereafter, sterile molten iron medium (Qingdao Hope Bio-Technology co. Ltd) was poured into the plates, swirled and allowed to set. The plates were incubated for 120 hours (5 days) at 37 ℃ to yield iron-related bacterial growth (Zakharova & Parfenova, 2007). Distinct colonies were picked off with flamed wire loop and were sub-cultured on fresh nutrient agar plates at 37 °C for 24 h. The cultural characteristics of the different colonies including colony form, colony elevation, colony margin, colony size, colony texture, color, opacity, surface and odor were observed and recorded. To aid in the identification of the pure isolates biochemical test which includes; gram stain, citrate utilization test, indole production test, catalase test and motility tests were performed (Spiegelman *et al*., 2005; Buchanan, 1974).
Determination of in vitro chlorine tolerance of isolates: A stock solution of calcium hypochlorite solution (5000 ppm) was prepared and dilutions of 1000 ppm, 100 ppm, 50 ppm, 10 ppm and 1 ppm were made. The test isolates were grown in peptone broth for 24 h. Thereafter, a serial dilution was made to an optical density of 0.2 at 600 nm. Cultures of isolated strains were inoculated into the test tubes containing the chlorine solution at different concentrations. The exposure of the isolates in the chlorine solution was varied from 5mins to 15 min. After the exposure time, the cells were inoculated into fresh nutrient agar plates and were incubated for 24 h at 37 °C. Plates without the growth of the isolates were recorded as susceptible (S) while plates with bacterial growth were recorded as resistant (R).

Figure 1: Sample site at Azikoro Village

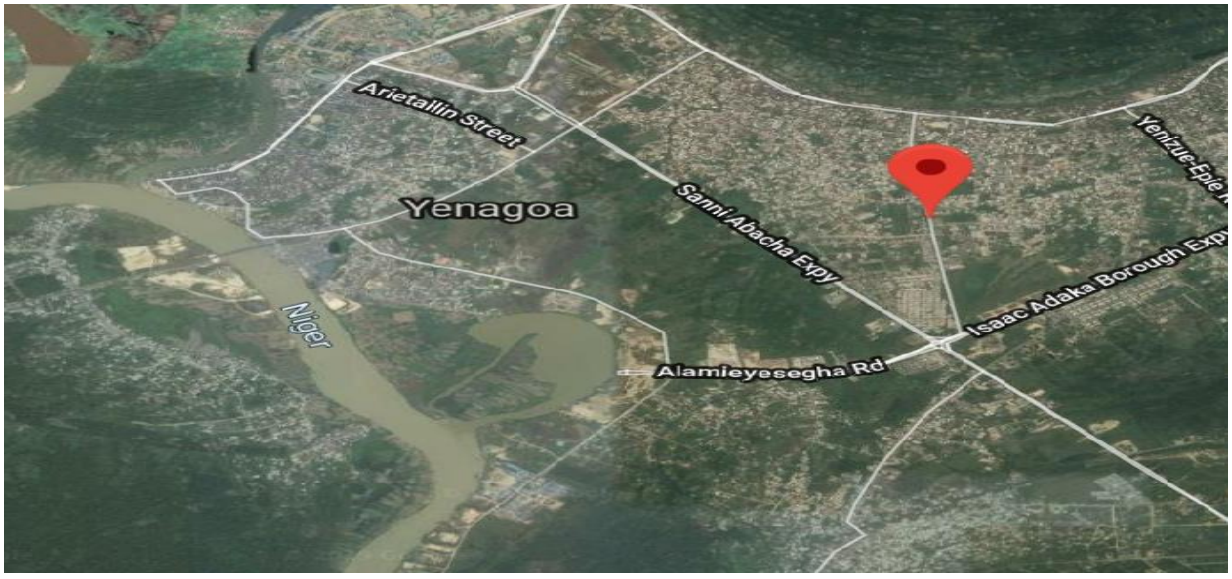

Figure 2: Study locations plotted on Google Earth image. The red circle on the map represents the locations/area where samples were collected in Yenagoa metropolis.

Molecular Analysis

DNA Extraction: DNA from pure cultures of iron-related bacterial isolates were extracted using Zymo Research Microbe DNA extraction Kit™ (Zymo Research Corp SA). The extracted DNA from the bacterial isolates were quantified using a NanoDrop ND- 2000 Spectrophotometer.

PCR Amplification of 16S rRNA: The extracted DNA was amplified using 16S rRNA universal primer set, 27F- (5'AGAGTTTGATCCTGGCTCAG-3') and 1492R- (5'CGGTTACCTTGTTACGACTT-3') on a 9700ABI thermal cycler. The PCR ingredients included Master mix, DNA template, Taq DNA polymerase, and PCR buffer. At a PCR condition of an initial heating step at 95 °C for 3 min, a total of 35 PCR cycles were run under the following conditions: denaturation at 95 °C for 30s, annealing temperature at 52°C for 30s and extension 72 °C for 1 min 30s and a final extension at 72 °C for 10 min. Amplified DNA was further resolved by electrophoresis in a 1% Agarose gel and viewed using a UV light transilluminator.

Sequencing: The amplicons were sent to South Africa and sequenced on a ABI 310 XL genetic analyzer using the ABI Big Dye Terminator cycle sequencing kit version 3.1 by Inqaba, South Africa. The obtained 16s rRNA sequence from the isolates were blasted on the NCBI database to obtain highly similar sequences.
Phylogenetic analysis: Obtained sequences were edited using the bioinformatics algorithm Trace edit, similar sequences were downloaded from the National Center for Biotechnology information NCBI) data base using BLASTIN. These sequences were aligned using MAFFT. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0 (Saitou and Nei, 1987). The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were computed using the Jukes-Cantor method. The phylogenetic and molecular evolutionary analysis was conducted using MEGA7.

Results and Discussion

Water is a universal solvent and groundwater usually has some characteristics of the soil and rock where it is found. Iron is very common in groundwater being one of the most abundant minerals in the earth crust. The presence of much iron in groundwater increases the abundance of iron bacteria. Iron bacteria are most likely to develop in wells and borehole systems. Although, over the years, iron bacteria have not been associated to any disease, their growth results in an increase in the organic content of the water that could in turn encourage the growth of other opportunistic organisms. The formation of insoluble hydroxides in the water formed when Fe2+ is oxidized to Fe3+ in the presence of oxygen (Soguard *et al*., 2003), as a matter of fact is what results in the problems of stain as could be seen in the overhead tanks where we collected water samples.
Four locations designated as (L1, L2, L3 & L4 respectively) in Yenagoa were studied. From each of the locations, three different points were randomly sampled twice (P1, P2, &P3). The results of the Fe content value and the total colony forming unit is as shown on Table 2. Location 1 (L1) recorded the least Fe content value (0.13- 0.31mg/l) and the highest iron bacteria population, the range is $3.2x10^4$ - $3.7x10^4$ CFU/ml. L2 recorded also very high values of iron bacteria particularly at P1 & P2 with a range of ($1.2x10^2$ – $3.4x10^4$CFU/ml) and very low iron bacterial value at P3 although with high values of iron content ranging from (0.44- 0.98mg/L). Although the values of iron bacteria recorded in L3 are significantly low (2.0x102 -$4.3x10^2$ CFU/ml), surprisingly, its iron content values were the highest (0.46-1.90mg/L). P2 & P3 samples of L4 were lost on transient. Except for P1 that has high Fe content values (4.0mg/L) with low iron bacteria value ($4.6x10^2$cfu/ml). In this study, the minimum and maximum iron concentrations of 0.13mg/L and 1.9mg/L respectively were observed in the water samples. The water samples have iron concentration above the permissible WHO standard value of 0.3mg/L (Oyem *et al*., 2014), the observed iron concentration above the permissible levels could be linked to dissolution of iron natural deposits into groundwater through leaching. This could put Yenagoa inhabitants at a risk of hemosiderosis/liver damage disease which is as a result of drinking water heavily laden with high iron concentration and arteriosclerosis (Brewer, 2009). The analysis of variance (ANOVA) indicates a positive significant difference in the population of the iron bacteria.
The sequences obtained from amplification of 16S rRNA gene gave a total of 8 different bacterial species capable of utilizing iron. The isolates showed a percentage similarity to other species at 97.5-100%. The bacterial isolates obtained includes; *Myroides odoratimimus* strain Q11, *Lysinibacillus xylanilyticus* strain EB-229, *Pseudomonas xiamenensis* strain B13, *Shewanella chilikensis* strain B0B2, *Janibacter indicus* strain YFY001, *Bacillus velezensis, Aeromonas hydrophila.subsp. Hydrophila* strain JCM3989, and *Staphylococcus sciuri* strain SNUDS-18. The identified bacterial sequences belonged to five phylogenetic groups; Proteobacteria, Firmicutes, Bacteroidetes, Flavobacterium, and Actinobacteria.
Among the bacteria isolated from the groundwater studied is *Aeromonas hydrophila.* Members of this genus are ubiquitous and are associated with the aquatic environment. *Aeromonas hydrophila* has been isolated from distribution Mains, Wells and Boreholes and is believed to be capable of causing gastroenteritis (Ratnayaka *et al*., 2009). *Aeromonas hydrophila* is considered as the best-known species of iron reducers (Woznica *et al*., 2003; Kooli *et al*., 2019). As a matter of concern, *Aeromonas hydrophila* is capable of causing infection in humans particularly, gastroenteritis. Also, the frequent isolation of *Aeromonas* from floodwater samples (Villari *et al*., 2003) suggests that this organism can pose public health concern since groundwater is the major source of drinking water in the Niger Delta. *Myroides odoratimimus* is a flavobacterium responsible for infrequent infections known for its multidrug-resistant and showing potential for biofilm formation. They are widely distributed in the environment especially in water, and are acquired as consequences of contact with contaminated water (Benedetti *et al*., 2011). *Myroides odoratimimus* isolation from our water sample should pose a public health concern since the people of Niger Delta rely on groundwater as their portable water.

Table 1: Enumeration of iron related bacteria population on Iron Bacteria medium

Samples (Boreholes ID)	TCFU/ml
L1 P 1	3.7×10^4
L1 P2	3.6×10^4
L1 P3	3.2×10^4
L2 P1	3.4×10^4
L2 P2	1.1×10^4
L2 P3	1.2×10^2
L3 P1	2.6×10^2
L3 P2	2.01×10^2
L3 P3	4.3×10^2
L4P1	4.6×10^2

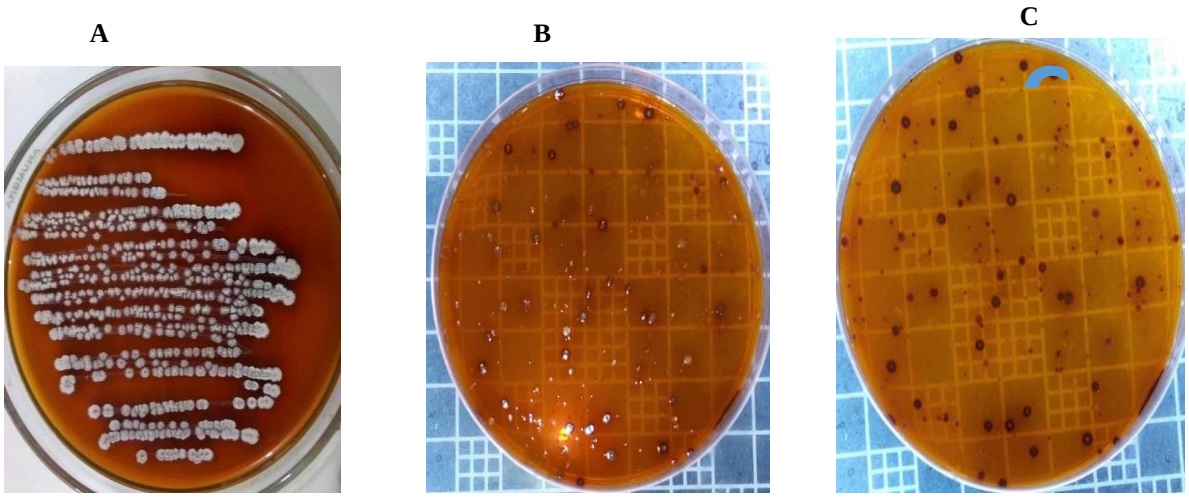

Figure 3: A, B, C of iron bacteria isolates from the water samples

Table 2: Bacteria isolates from the three different locations

Locations	Region	Samples ID	Fe /mg/L Conc	TCFU/ml	Isolates ID/ Location	Isolates from different locations
1	Azikoro	L1P1	1.74	$3.7x10^4$	B1	*Myroides odoratimimus*
	Town	L1P2	1.90	$3.6x10^4$		
		L1P3	0.46	$3.2x10^4$	B2	*Lysinibacillus xylanilyticus*
2	Swali	L2P1	0.58	$3.4x10^4$	B3	*Pseudomonas xiamenensis*
	Community	L2P2	0.98	$1.14x10^4$		
		L2P3	0.44	$1.16x10^2$	B4	*Shewanella chilikensis*
3	Kpansia	L3P1	0.13	$2.6x10^2$	B5	*Janibacter indicus*
	Community	L3P2	0.36	$2,0x10^2$		
		L3P3	0.24	$4.3x10^2$	B6	*Bacillus velezensis*
4	Yenezue	L4P1	1.4	$4.6x10^2$	B7	*Aeromonas hydrophila*
	Gene	L4P2	-	$2.5x10^2$		
		L4P3	0.30	$2.12x10^2$	B8	*Staphylococcus sciuri*

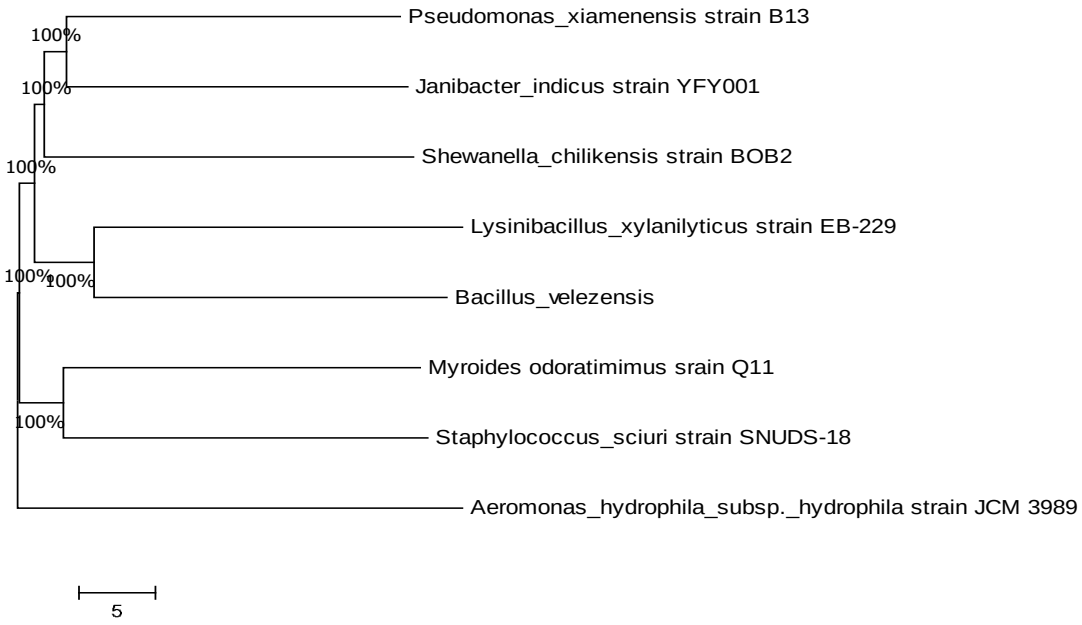

Figure 4: Phylogenetic analysis of the bacterial isolates

Table 3: Total iron bacterial count (CFU/ml) in response to increasing chlorine concentration (ppm) and time (min).

	Conc. (ppm)	**1**		**10**		**50**		**100**		**1000**		**5000**	
	Time (min)	**5**	**15**	**5**	**15**	**5**	**15**	**5**	**15**	**5**	**15**	**5**	**15**
ISOLATES	**B1**	1.90 x 10^3	1.76 × 10^3	1.54 × 10^3	1.32 × 10^3	1.12 × 10^3	1.00 × 10^3	8.1 × 10^2	6.4 × 10^2	3.0 × 10^2	***	-	-
	B2	1.24 × 10^3	1.02 × 10^3	9.2 × 10^2	8.2 × 10^2	8.0 × 10^2	7.8 × 10^3	5.0 × 10^2	4.1 × 10^2	***	***	-	-
	B3	1.70 × 10^3	1.52 × 10^3	1.21 × 10^3	1.12 × 10^3	1.02 × 10^3	9.4 × 10^2	6.2 × 10^2	5.0 × 10^2	4.2 × 10^2	***	-	-
	B4	1.58 × 10^3	1.38 × 10^3	1.32 × 10^3	1.14 × 10^3	9.6 × 10^2	8.4 × 10^2	5.8 × 10^2	4.9 × 10^2	3.3 × 10^2	***	-	-
	B5	1.74 × 10^3	1.55 × 10^3	1.23 × 10^3	1.02 × 10^3	8.7 × 10^2	8.0 × 10^2	6.8 × 10^2	4.8 × 10^2	3.5 × 10^2	***	-	-
	B6	1.81 × 10^3	1.64 × 10^3	1.40 × 10^3	1.31 × 10^3	1.00 × 10^3	8.1 × 10^2	5.3 × 10^2	4.3 × 102	3.1 × 10^2	***	-	-
	B7	1.32 × 10^3	1.16 × 10^3	9.1 × 10^2	8.6 × 10^2	7.7 × 10^2	6.8 × 10^2	4.5 × 10^2	3.9 × 10^2	***	***	-	-
	B8	1.68 × 10^3	1.38 × 10^3	1.01 × 10^3	9.1 × 10^2	8.0 × 10^2	7.1 × 10^2	4.8 × 10^2	4.0 × 10^2	***	***	-	-

Key: (*******) colony number less than 25 **(-) No iron bacteria growth

This study also attempted to ascertain chlorine tolerance levels of the identified iron bacteria strains. The chlorine tolerance of the isolated iron related bacteria to different concentrations of chlorine at different contact times, corresponding to the bacteria strains are shown in Table 3. It was shown that all the iron bacteria strains were able to grow in chlorine concentrations of 1 ppm, 10 ppm, 50 ppm, 100 ppm and 1000 ppm for 5 and 15 min. However, we observed that the colony forming unit obtained at the different concentrations of chlorine and contact times, beginning from 1ppm at 5min differed between the bacterial strains. The growth of the iron bacteria strains was totally inhibited at chlorine concentration of 5000ppm. Although the level of chlorine tolerance varied between the isolates, the results obtained suggests that the all the iron utilizing bacteria identified in this study can tolerate high concentrations of chlorine. In a similar experiment by Mohammed *et al.,* (2014), it was suggested that the differences in chlorination resistance could be attributed to the encapsulation or concentration of nutrients in the media. They further reported that either the formation or the modification processes of membrane accessory components involved in protein synthesis, and on the other hand, unique proteins are synthesized by bacteria as response to stress, and this can enhance resistance. In a different study, Martin (2012), posited that bacteria can synthesize unique proteins as a response to stress, which can contribute to resistance and that the composition of the bacteria cell wall also influences its resistance to adverse environmental conditions and stress such as chlorination.

Our Investigations into the nature of mechanisms in the bacterial isolates used in this study reveals they possess different properties that could enhance resistance to chlorination. Regarding the mechanisms for withstanding the cidal effect of chlorine, *Myroides spp*. have been reported to possess a polysaccharide capsule, which makes the bacterial surface to be extremely hydrophobic (Lorenzin *et al.,* 2018). Also, *Lysinibacillus xylanilyticus* which is a gram positive, aerobic, motile bacterium possess an endospore. *Janibacter spp* also produces endospore. Similarly, encapsulation has been reported in *Aeromonas hydrophila* and S*taphylococcus spp* (Villari, 2003). Iron reducing bacteria such as *Shewanella* have previously been studied with regard to their cell surface ultrastructure and lipopolysaccharide composition (Korenevsky et al., 2002). Shewanella plays important roles in the geochemical cycling of iron oxides. (Korenevsky et al., 2002; Loveley *et al.,* 2004). *Pseudomonas xiamenensis* strain B13 recorded the highest plate count of 4.2×10^2 tcfu. This implies that isolate B3 may be more resistant to chlorination than other the strains examined in this study. No significant growth (>25) however was recorded in the same concentration (1000ppm) at 15 minutes' exposure time. The second most resistant strain was isolate B5- *Janibacter indicus* strain YFY001, followed by isolate B4- *Shewanella chilikensis* strain B0B2

A correlation analysis between the chlorine concentration and the time of exposure of the bacterial isolates was done using statistical package for social sciences (SPSS). The analysis indicates different significant relationships between the contact time and level of chlorination. Strain B1 recorded a positive and significant correlation at 5 minutes and 15 minutes, with p values of 0.01 and 0.001 respectively. Similar significant and positive relationships was recorded in B3 and B4. However, there was no positive and significant relationship between the concentration of chlorine, the time of exposure and the cfu/ml obtained in the other isolates. This may suggest that other factors beyond the concentration of chlorine in the growth medium and the contact time may have influenced the rate of growth and survival of the iron bacterial isolates. However, this study investigated just the degree of influence of the concentration level of chlorine and contact time on the survival and growth of the iron bacterial isolates.

The phylogenetic analysis of the isolated iron bacteria examined in this study is as shown in Fig 4. The evolutionary distance between a pair of sequence usually is measured by the number of nucleotide substitutions occurring between them. The sequences are compared nucleotide by nucleotide. The bootstrap for the branches were 100%. As a general rule, if the bootstrap value for a given interior branch is 95% or higher, then the topology at that branch is considered correct (Nei and Kumar 2000). Also, the composition distance in the 8 sequences was done. The analysis indicated there is an overall average of 10.173 differences.

The evolutionary relationship was inferred using the neighbor joining method. As shown in Fig 4, the *Pseudomonas xiamenensis* B13, *Janibacter indicus* YFY001, and *Shewanella chilikensis* B0B2 are closely related as they share the same clade. However, *Pseudomonas xiamenensis* and *Janibacter indicus* are more closely related, as they share a common node (ancestor).

In this study, we observed the phylogenetic relationship expressed in the pattern of chlorine resistance. The high chlorine tolerance of these most related strains as shown in the phylogenetic tree may be as a result of the presence of defense mechanisms, they exhibit to withstand toxic chemicals like chlorine. More so, *Shewanella chilikensis* and *Pseudomonas xiamenensis* bacteria have been reported to produce polysaccharide capsules which may confer on them, the ability to resist certain chlorine to certain degree.

The phylogenetic tree shows these two strains share a common node (ancestor) with a very similar branch length of 23.2500 and 23.7500 respectively. It is however worthy to note that the phylogenetic tree suggests that the *Aeromonas hydrophila* JCM3989 seemed to be the ancestral to the other strains isolated in this study. Table 3

shows that the *Aeromonas hydrophila* JCM3989 recorded no significant growth at 1000ppm chlorine concentration, for 15minutes contact time.

The *Staphylococcus sciuri* SNUDS-18 and the *Myroides odoratimimus* Q11 which had very poor survival rate in the chlorinated medium are placed in the same node, in the phylogenetic tree. With keen observation of the phylogenetic relationship between the isolated bacteria strains, we observed the highly resistant ones are placed together, usually originating from the same node, like the *Pseudomonas xiamenensis* B13, *Janibacter indicus* YFY001 and *Shewanella chilikensis* B0B2 while the other strains that exhibited low tolerance to chlorine such as the *Myroides odoratimimus* Q11, *Staphylococcus sciuri* SNUDS-18 and *Aeromonas hydrophila* JCM3989 are more related. In fact, the tree shows *Aeromonas hydrophila* strain JCM3989 to be the ancestral strain. We can therefore suggest that the level of resistance to chlorine may be as a result of evolutionary changes/mutations in the nucleotide sequence of the bacterial species that ensures their survival in extreme environments. As such, we posit that the resistance potential may evolve in these bacteria with time.

The presence of iron bacteria in the water causes aesthetic problems as indicated by rusty slime inside overhead tanks observed almost on all overhead tanks in the Niger Delta region, reduced water flow from the borehole and unpleasant taste and odour. The most common indication of iron bacteria in the water supply is a reddish-brown or yellowish gelatinous slime in water tanks, faucets, toilet tanks, and plumbing. These nuisance bacteria may cause corrosion to treatment equipment, clog screens and pipes. The growth of iron bacteria also results in increase in organic content of the water and could in turn encourage the growth of other nuisance organisms.

Conclusion

The impacts of iron bacteria have been a daunting challenge to most societies, as the effects are still observed after water treatment. This study provides a reason for the survival of iron bacteria after water treatment. It has been shown that iron bacteria isolated in this study were resistant to chlorine even at 1000 ppm, we therefore, recommend that an appropriate higher dose of chlorine treatment be used in water treatments. Based on the finding provided by our study, we recommend that there should be intentional and systematic efforts to analyze the effects of seasonal variation on the physiochemical parameters of groundwater and their relationships with iron bacteria population. Also, since the presence of iron in water promote the proliferation of iron bacteria, then, there should be conscious effort to reduce the iron content of groundwater especially, in reservoir tanks, as a measure of inhibiting the rate of proliferation of iron bacteria.

References

Akpokodje EG: A method of reducing the cement content of two stabilized Niger Delta soils. Q J Eng Geol Lond. 19:359–363. 1986.

Benedetti P, Rassu M, Pavan, G: Septic shock, pneumonia, and soft tissue infection due to *Myroides odoratimimus*: report of a case and review of *Myroides* infections. Infection 39: 161-165. 2011.

Brewer,G: Risks of Copper and iron toxicity during aging in humans. Chem. Res. Toxicol 2: 319-326. 2009.

Bruce Rauner G: Iron in Drinking water. *Environmental Health (Illinois Department of Public Health* http://www.idph.state.il.us/envhealth/factsheets/ironFS.htm. 2010.

Buchanan RE, Gibbons NE: (eds) *Bergey's Manual of Determinative Bacteriology*, 8th Elm. *Baltimore:* Williams & Wilkins Co., Baltimore, MD 21202. Xxvi + 1246 pp. 1974.

Chapelle FH: *Ground-Water Microbiology and Geochemistry*. John Wiley and Sons Inc, New York. 468 p. 2000.

Ratnayaka DD, Brandt MJ, Johnson KM: Disinfection of Water in Water Supply (Sixth Edition). Butterworth-Heinemann. Boston. pp. 109-147. 2009. doi.org/10.1016/B978-0-7506-6843-9.00012-3.

Ehrlich HL, Newman DK: eds. *Geomicrobiology, Fifth Edition.* CRC Press, Boca Raton, FL. 2009. ISBN 9780849379062. https://resolver.caltech.edu/CaltechAUTHORS:20170522-092432123

Kooli WM, Junier T, Shakya M, Monachon M, Davenport KW, Vaideeswaran K, Vernudachi A, Marozau I, Monrouzeau T, Gleasner CD, McMurry K, Lienhard R, Rufener L, Perret JL, Sereda O, Chain PS, Joseph E, Junier P: Remedial treatment of corroded iron objects by environmental aeromonas iIsolates. Appl Environ Microbiol. 85(3): 2019. e02042-18. https://doi.org/10.1128/AEM.02042-18

Korenevsky AA, Vinogradov E, Gorby Y, Beveridge TJ: Characterization of the lipopolysaccharides and capsules of *Shewanella* spp. Appl Environ Microbiol. 68(9): 4653-4657. 2000.

Lorenzin G, Piccinelli G, Carlassara L, Scolari F, Caccuri F, Caruso A, Antonia De Francesco A: *Myroides odoratimimus* urinary tract infection in an immunocompromised patient: an emerging multidrug-resistant micro-organism. Antimicrob Resist Infect Control. 7(96). 2018. https://doi.org/10.1186/s13756-018-0391-4

Lovely DR, Holmes DE, Nevin KP: Dissimilatory Fe(III) and Mn(IV) reduction. Adv Microb Physiol. 49: 219–286. 2004.

MacDonald AM, Calow RC: Developing groundwater for secure water supplies in Africa. Desalination, 248: 546-556. 2009.

Martins SCS, Faheina Junior G da S, Machado B de M, Martins CM: Chlorine and antibiotic-resistant bacilli isolated from an effluent treatment plant - doi: 10.4025/actascitechnol.v35i1.12951. Acta Sci Technol, *35*(1):3-9. 2012. https://doi.org/10.4025/actascitechnol.v35i1.12951

Mohammad IA, Zubair AI, Bayazed H: Assessment of chlorine resistant bacteria and their susceptibility to antibiotic from water distribution system in Duhok province J Appl Biol Biotechnol. 2(06): 010-013. 2014.

Nei M, Kumar S: *Molecular Evolution and Phylogenetics*. Oxford University Press, Oxford. pp. 333. 2000.

Okiongbo KS, Oboshenure KK, Amakiri ARC: Investigation of lithological control of iron enrichment in groundwater using geophysical techniques in Yenagoa, Southern Nigeria. SN Appl Sci 2(1) 107. 2019. https://doi.org/10.1007/s42452-019-1876-3.

Oyem HH, Oyem IM, Ezeweali D: Temperature, pH, electrical conductivity, total dissolved solids and chemical oxygen demand of groundwater in Boji-Boji Agbor/Owa area and immediate suburbs. Res J Environ Sci. 8(8): 444-450. 2014.

Popoola LT, Yusuff AS, Aderibigbe TA: Assessment of natural groundwater physicochemical properties in major industrial and residential locations of Lagos metropolis. Appl Water Sci. 9:191. 2019.

Saitou N, Nei M: The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 4: 406-425. 1987.

Sogaard EG, Aruna R, Abraham-Peskir J, Koch CB: Conditions for biological precipitation of iron by *Gallionella ferruginea*, in a slightly polluted groundwater. Appl Geochem, 16: 1129-1137. 2003.

Spiegelman D, Whissell G, Green CW: A survey of the methods for the characterization of microbial consortia and communities. Can. J. Microbiol. 51:355-386. 2005.

Stuart AS: Primer on Microbial Problems in Water Wells. https://groundwaterscience.com/resources/tech-article-library/96-primer-on-microbial-problems-in-water-wells.html. 2015. Accessed July 21, 2021.

Szewzyk U, Szewzyk R, Manz W, Schleifer KH: Microbiological safety of drinking water. Annu. Rev. Microbiol. 54:81-127. 2000.

Villari P, Crispino M, Motuori P, Boccia S: Molecular typing of *Aeromonas* isolates in natural mineral waters, Appl Environ Microbiol, 69(1): 697-701. 2003.

World Health Organization (WHO): Guidelines for drinking-water quality. 2nd ed. Geneva. http://www.who.int/water_sanitation_health/. 1993.

Woznica W, Dzirba J, Manka D, Labuzek S: Effect of electron transport inhibitor on iron reduction in *Aeromonas hydrophila* strain KB1. Anaerobe 9: 125-130. 2003.

Younger PL: *Groundwater in the Environment: an Introduction*. Blackwell: London. 390 p. 2007.

Zakharova YR, Parfenova VV: A method for cultivation of microorganisms oxidizing iron and manganese in bottom sediments of Lake Baikal. Biol Bull. 34:236-241. 2007.