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Research article

Characterization of iron Bacterium *Gallionella ferruginea* isolated from the drinking water of the collector wells in Northern Sri Lanka

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Abstract: The objective of the study was to isolate, identify and characterize the organism/s responsible for the brownish slime colour and bad odour of the water from the collector wells at Vallipuram area of the Northern Sri Lanka. The iron concentrations in the collector well samples that are free of fecal coliform bacteria, varied from 0.093 to 0.307 mg.l⁻¹ during the day time. Based on the microscopic biochemical and molecular characterization, the bacterial strain isolated from the collector wells was identified as *Gallionella ferruginea*. They are gram negative kidney-shaped mycoplasmodial bodies found in clusters. The colonial growth was powdery, opaque and flat in elevation. The biochemical characterization showed the positive interpretation for indole and catalase tests while methyl red, citrate, Voges-Proskauer, urease production, nitrate reduction, tyrosine utilization, acetoin production and oxidase tests showed negative. The bacteria were capable of fermenting glucose with the production of acid in anaerobic condition, but not in aerobic condition thus confirmed as *Gallionella ferruginea*. This bacterial strain grew well in iron added liquid media at temperatures between 25–40°C and the optimum growth was observed at 35°C. Though *Gallionella* grew well at a broad range pH values between 6.0–10.0, the optimum growth was obtained at neutral pH. Addition of NaCl in the iron added liquid media can inhibit the growth and multiplication of *Gallionella ferruginea*.

Keywords: Brownish slime - Water quality - Iron bacteria - *Gallionella ferruginea* - Vallipuram.

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INTRODUCTION

Water is one of the principal components in determining the quality of our live and the living environment. Although water covers about 70 percent of the earth's total surface, only 0.3% of it can be utilized by humans (Tiimubet *et al.* 2012). Jaffna Peninsula of Sri Lanka has a low percentage of surface water sources because of its karstic nature and flat terrain (Meisler 1977, Dissanayake & Senaratne 1981, Senaratne & Dissanayake 1982, Wijesekera *et al.* 2012, Hidayathulla & Karunaratna 2013). Group of aerobic bacteria that can utilize the oxidation of ferrous or manganous ions as an essential element in their metabolic utility are defined as iron bacteria. The resultant production of ferric acid or manganic salts within the cell or cell coating gives the bacteria their typical brown coloration. Studies of pyrite deposits have shown them to contain a range of fossil bacteria including representatives of two major groups of iron bacteria, *Gallionella* and *Sphaerotilus* (Schopf *et al.* 1965). Iron can be deposited as filamentous amorphous gelatinous type of brown-reddish slime that precipitates from water that contains iron. Iron deposits have become a serious issue in a variety of water saucers including drippers and could lead to plugging of a low volume system. There is a class of bacteria that can absorb ferrous ion and interchange it to a slimy matrix of bacterial bodies and convert into ferric ion. The complication typically exists in well water areas where the earth water aquifers are formed mainly of sandy soils

normally with a pH of down from 7.0 and in the absence of dissolved oxygen. These ground waters contain ferrous ion (Fe^{2+}) which is chemically reduced, completely water soluble and which serves as the starting raw material for slime production. The dissolved ion may precipitate out of the water due to differences in pressure and temperature, an increase in pH, electro conductivity or through the action of bacteria. The result is a sludge or slime that may reduce system performance.

The encrustations of wells are caused by iron and manganese bacteria, various species of which exist in the soil and could presumably enter a well during the initial boring operations or by seepage into the aquifer feeding the well (Hasselbarth & Ludemann 1972). The existence and morphology of *Gallionella ferruginea*, one of the important iron bacteria living in diverse aquatic environment, have been subjected for continuous discussion. Litho autotrophic metabolism of these bacteria has been questioned (Jarrgensen 1989). *Gallionella ferruginea* generally grows to a very low cell number, approximately 1×10^6 cells ml^{-1} , in the appropriate liquid medium (Hallbeck & Pedersen 1990). Iron bacteria of the well water react with the ferrous (Fe^{+2}) during the oxidation process and converts it to insoluble ferric (Fe^{+3}). This Ferric ion is enveloped by the filamentous bacterial colonies and forms the sticky iron slime gel that is responsible for clogging the water movement. The ferrous ion concentration low as 0.15–0.22 ppm is considered as a potential hazard to drip systems (Ford 1982). Between 0.2–1.5 ppm, of ferrous can cause clogging hazard in a moderate level. Concentrations above 1.5 ppm can cause severe blocking (Bucks & Nakayama 1980). Water resources that contain concentrations higher than 0.5 ppm of iron, should be considered for immediate chemical treatment before using filter. Chlorination can successfully control iron slime when iron concentrations were less than 3.5 ppm and the pH was below 6.5 (Nakayama & Bucks 1986). Ferrous ion in the water stream could be mixed with the wastewater that has an organic load associated with it. Microbial corrosion is an electrochemical process where microorganisms are able to initiate, facilitate or accelerate corrosion reaction without changing its electrochemical nature (Dexter *et al.* 1991, Iversen 2001).

Jaffna lagoon of the northern province of Sri Lanka is a shallow water body and has extensive mudflats, sea grass beds and some mangroves. Most of the wells that are closer to sea are not used for public consumption because of the salty in nature (Sayanthan *et al.* 2015, Kapilan 2015). It was decided to analyze its ground water since the ground water is collected and purified by the addition of chemicals and this processed water is distributed for public consumption. The occurrence of iron in geological strata of this Island is already well established. Because of the limited sources available and the difficulty of monitoring the organisms, limited attention has been paid to identify the organism and its role in the strata or aquifers in the change of water colour and the bad odour is not well understood. Soil, water and mud could be a habitat for the growth of diverse iron bacteria. Dissolved organic materials of the groundwater may be de-oxygenated by microbes feeding on that dissolved organic material. In water-supply wells, iron bacteria derive the energy they need to live and multiply by oxidizing the dissolved ferrous ion (Andrews *et al.* 2013). These iron bacteria causes problems in mining operations, piped water and sewage systems and in open bodies of water. The colour change and brownish slime formation would be always possible and the water becomes undrinkable and further growth would lead to plugging of freshwater wells. The problem continued and the Iron bacteria also formed and the bad hydrogen sulphide odour was observed. Sulfurous smell of rot or decay results from enzymatic conversion of soil sulfates to volatile hydrogen sulfide as an alternative source of oxygen in anaerobic environments that are used to collect water for drinking purpose. In the collector wells from where the drinking water is supplied to public, the major problems are, growths plugging the screens, coating of the pipes, impellers and motors, reduction in the water flow rates reduced portability of the water and all these may lead to total plugging of the well. However there is no recent scientific water analysis conducted for the collector wells to determine the water quality and to identify the potential pollutants specially the iron bacteria in the northern province of Sri Lanka so that some remedies for improvement could be possible. Vallipuram area that supplies water to the public for consumption through the collector well system was selected as an ideal place of the above mentioned situation. Therefore this study was aimed at determining the iron bacteria that is responsible for the bad odour and brownish slimecolour of the water in the drinking water collector wells in the Vallipuram area of the northern Sri Lanka.

MATERIALS AND METHODS

Study Area

This study was conducted in the Vallipuram costal area between September and December in 2015. Collector wells are located in the sand dunes. The location is rich in water and there are less hardness issues, less population density in the area so that anthropogenic activity is also very less.

Sampling method

Samples were collected randomly from four collector wells. From each of the water sources samples were collected in two positions one from top and other from bottom and used for the analysis. Standard precautionary measures were adopted to minimize cross contamination of samples. Sterilized plastic containers and Durant bottles were used for collecting water from collector wells. This was done carefully to avoid contact between the containers and the walls of the wells, thus avoiding contamination of samples. Samples were labelled as Sample 01: collector well 04 water sample, Sample 02: observation well - water sample, Sample 03: collector well 02 water sample, Sample 04: collector well 03 slime and water sample, Sample 05: collector well 03 water sample and Sample 06: collector well 01 water sample. The samples were carefully transported and stored at 4°C in the laboratory refrigerators and later used for the physico-chemical analysis based on SLS and APHA guidelines and microbiological and molecular biological studies.

Design of sampler

The sampler was made of stainless steel cylinder of 300 ml capacity. The upper end of the cylinder was sealed but contained three holes through which the water can enter into the cylinder. A movable piston was set up inside the cylinder and connected with the upper part of the sampler. Two long running threads were tied each on the middle of the cylinder as well as the piston. The thread fixed to the piston was used to bring down the sampler to a desired depth. Once the sampler reached to a desired depth the thread fixed to the piston was released by tightening the other thread, which was fixed to the cylinder. After a few minutes again the thread fixed to the piston was tightened, while releasing the other and the sampler was taken out from the well.

Optimization of growth conditions

The optimum temperature and NaCl concentration required for the growth of the bacterial isolate was determined. For determination of optimum temperature, 0.1 ml of freshly prepared suspension of the bacterial isolate was inoculated in 10 ml of iron added liquid media and incubated at different temperatures ranging from 0–65°C. After an incubation period of 72 hr, the growth was checked in the form of turbidity and by measuring the OD at 600nm. Similar experiment was done with different concentrations of NaCl ranging from 0–50g.l⁻¹ while incubated at 35°C in the iron added liquid media. Similar experiment was done with different pH values of the media ranging from 4.0–12.0. Different buffer solutions were used to maintain the buffer of the liquid media constant throughout the experiment.

Morphological and biochemical characterization of isolates

Colony morphology such as form, elevation, margin, opacity, diameter after 40 h growth (in mm), colour and surface of the strain, were studied to identify the Genus of the isolated strain. Shape and arrangement of endospore were observed under oil-immersion microscope after gram staining. Production of acid from different carbohydrates such as glucose, xylose and mannose were tested. Production of urease, hemolysis of blood agar, indole test, nitrate reduction test, decomposition of tyrosine, hydrolysis of starch, citrate utilization test and Voges-Proskauer (VP) test were done on the isolated strain (Barrow & Feltham 1993, Fisher 1975, Theivendrarajah 1990). Growth of the selected strain was tested at 5, 15, 25, 35, 40, 45, 50, 55 and 60°C at pH 9.0, and 100 rpm. The bacterial strain that was isolated from the water sample was subjected to gram staining (Kaiser 2001) and motility test by hanging drop (Theivendrarajah 1990). Oxygen requirement test, test for anaerobic growth, Catalase test, Oxidase test, Triple Sugar Iron Agar test and Lactose Fermentation test were done on the isolated strain (Kapilan & Arasaratnam 2010, Theivendrarajah 1990).

Molecular Identification of bacterial isolate

The bacterial isolate was sent for identification by 16S rRNA sequencing at NCCS, India. The 16S rRNA gene sequence of the isolate was aligned with reference 16S rRNA gene sequences of the European Microbiological Laboratory (EMBL), GeneBank and the database of Japan using the BLAST algorithm available online (<http://blast.ncbi.nlm.nih.gov/>).

RESULTS AND DISCUSSION

Iron is found to be dissolved in water and when brought to the surface, it can form rust (Goonetilleke *et al.* 2015). Iron-reducing bacteria add iron to the water by attacking the piping of the system. Removal of natural iron in the water requires special water treatment equipment (Hamilton 1985, Trivedy & Goel 1986). Multiplication and spread of iron-reducing bacteria could be controlled by adequate chlorination. The most common features that could be visible because of the colonization of iron bacteria in the water are appearance of reddish water, spotting, metallic taste of water and staining of plumbing fixtures. These features are possible when the concentration of iron in the water is more than 0.3 mg.l^{-1} . The smells of most of the water of the collector well is like irony. Irony smell may be due to the corrosion of iron pipes.

Morphological characterization of isolates

Identification of genus of the isolated strain

To identify the species of the isolated strain, diverse guides (Chessbrough 1984, Theivendrarajah 1990) were used. Tests on ground waters in Vallipuram area have been 95% positive for the presence of iron bacteria and microscopic examination of the surface growths has revealed the dominant types to be *Crenothrix*, *Leptothrix*, *Sphaerotilus* and *Gallionella*. The cells possess a long spirally twisted stalk arising from centre of cell. The bacterium is a kidney-shaped mycoplasmodial cell body. This body has a single elongated stalk that is made from "numerous helically wound, uniquely mineralized fibrils outward from the convex side". These stalks are generally covered in bacteriogenic iron oxide precipitate which gives it a reddish-brown colour (Anderson & Pedersen 2003). After 40 h of growth, the white colour colony with a diameter between 1.5 and 2.0 mm was observed. Based on studies so far carried out, it can be confirmed that the isolated strain belongs to the genus *Gallionella*. Its species has been identified, using the following experiments.

Morphological Characteristics

Gram staining showed gram negative kidney shaped (curved) and found in clusters (Fig. 1). But these curved cells had a sheath on the outer surface which appeared lightly stained. The growth was powdery, opaque and flat in elevation (Fig. 2). The bacterium *Gallionella* is generally a kidney-shaped mycoplasmodial cell body. This body has a single elongated stalk that is made from numerous helically wound, uniquely mineralized fibrils outward from the convex side (Anderson & Pedersen 2003, Halbach *et al.* 2001). Growth of the *Gallionella* sp. was observed only in the iron agar media. When a loopful of the enriched broth added with iron was isolated on the same solidified medium, a pale yellow coloured growth was observed on the plates after an incubation of 72 hours. Individual separate colonies were also obtained. Since only one type of growth was observed hence, it could be concluded that it is possible to obtain the isolate as a pure culture. Since the iron agar media tend to dry up very fast even after refrigeration, frequent sub-culturing was performed.

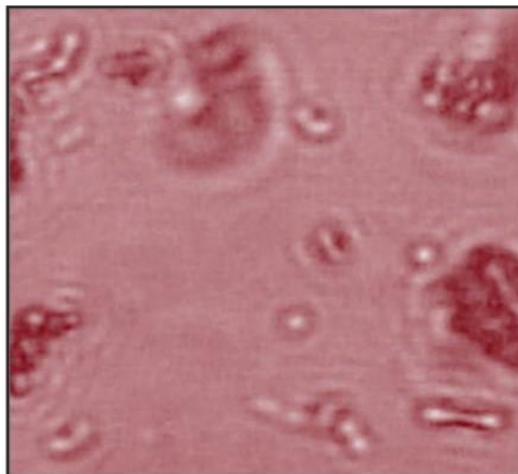


Figure 1. Microscopic view of bacterium *Gallionella ferruginea* growing on the iron agar media (Kidney shaped cells).

Determination of species of the isolated strain

When serum-water glucose medium, serum-water xylose medium and serum-water mannose medium were inoculated with the strain and incubated at 37°C for 24 h, the colour changed to red with water glucose medium. This indicated that the strain can produce acid from glucose but not from xylose and mannose. If the strain is a urease-producer, the enzyme will hydrolyze urea by releasing ammonia and carbon dioxide. Ammonia released will change the phenol red indicator to red pink colour due to the change in pH of the medium to alkaline

conditions. Medium inoculated with strain did not show any colour change. This indicated that strain is not a urease producer. When blood agar plate was inoculated with strain, around the bacterial colony a transparent clear zone was not observed. This indicated that the strain has no capacity to break down the haem in hemoglobin present in blood. When tryptophan is metabolized by an organism using the enzyme tryptophanase, indole is produced and the presence of indole can be detected using Kovac's reagent. Kovac's reagent is yellow in colour. It contains 4(p)-dimethylamino-benzaldehyde, which reacts with indole and produces a red colour on the surface of the reagent. Light red or pink colour at the border of Kovac's indole reagent and tryptophan solution (tryptone water) indicates the formation of indole (Theivendrarajah 1990). When tryptone water medium was inoculated with the isolated strain and mixed with Kovac's indole reagent, red colour ring was observed. This indicated that strain can utilize tryptophan and produce indole.

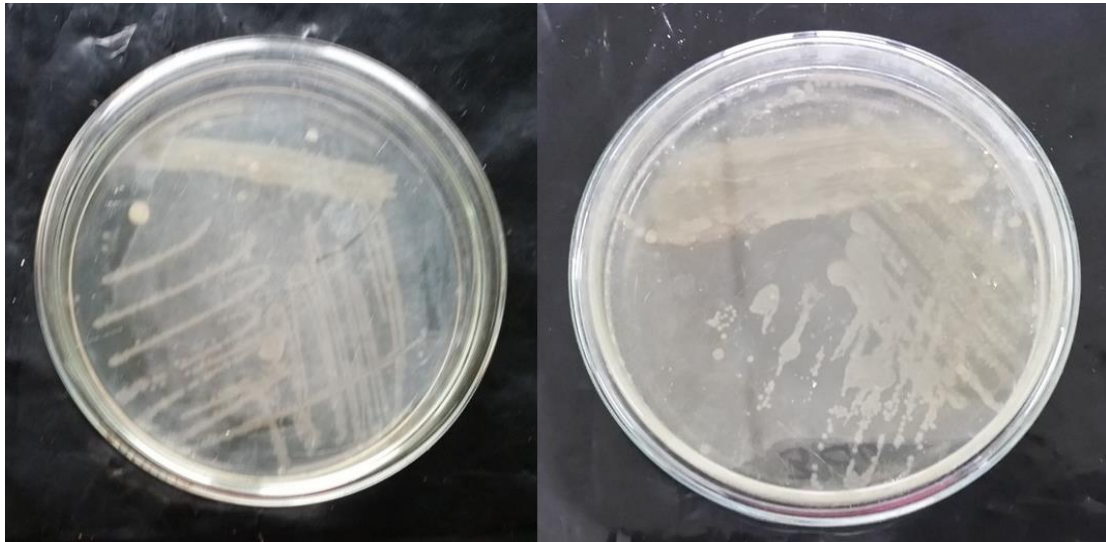


Figure 2. Colony morphology of the bacterium *Gallionella ferruginea* while growing on the iron agar media.

Reduction of nitrate to nitrite by an organism can be tested by incubating one loopful of inoculum in a broth containing nitrate and after 4 h adding sulphanic acid reagent to the broth. If nitrite is present the acid reagent is diazotized and forms a pink-red compound with alpha naphthalamine. The strain did not show any colour change, indicating that it does not have the ability to produce nitrate reductase. If the organism decomposes tyrosine crystals there should be a clear zone under and around the bacterial growth. When the tyrosine-agar plates were inoculated with strain on the iron agar plate, no clear zone was observed around and under the colonies. This indicated that the strain did not utilize tyrosine. If the organism produces starch hydrolyzing enzymes it can hydrolyse starch into monosaccharides. To check whether the organism has utilized starch, after the growth of the organism I₂ has to be added. If there is blue colour formation, it indicates the production of starch hydrolyzing enzyme (Theivendrarajah 1990). When starch-agar medium was inoculated with the strain and I₂/KI was added after 48 h of incubation, a blue colour was formed. This indicated that the strain did not produce starch-hydrolyzing enzymes.

Bacteria that utilize citrate can extract nitrogen from the ammonium phosphate incorporated in the medium, resulting in the production of ammonia, which combines with water to form NH₄OH. These reactions in combination produce an alkaline pH, resulting a colour change in the indicator from green to blue. If the organism does not utilize citrate, the medium remains green in colour. When citrate phosphate broth was inoculated with the strain, the medium remained green. This indicated that the bacterial strain did not utilize citrate as the carbon source. If acetoin is produced by the organism, in the presence of sodium hydroxide acetoin is oxidized to diacetyl. The diacetyl will give a pink compound with creatine. The isolated bacterial strain was cultured in glucose-phosphate-peptone water and incubated at 37°C for 48 h. When creatine and sodium hydroxide were added the culture and exposed to air, no pink colour was obtained. This indicated that the strain has no ability to produce acetoin by the fermentation of glucose.

Microscopic studies and Biochemical tests

Biochemical tests were carried out to confirm the genus of the strain and to identify the species. The bacterial strain had shown good growth both under aerobic condition and anaerobic condition (in anaerobic jar).

This indicated that the strain is mostly anaerobic. The strain could not produce O₂ from H₂O₂. This showed that the strain is not a catalase producer. If the bacterium oxidizes tetramethylp-phenylenediaminedihydrochloride, it will turn purple, indicating that the organism can produce cytochrome oxidase. No change in colour indicates that there is no production due to lack of cytochrome oxidase. The strain did not bring about a colour change. Therefore, it does not produce cytochrome oxidase. The strain ferment lactose, sucrose and glucose. The strain inoculated slants showed pink red butt and yellow slope. These results indicated that the bacterial strain ferments glucose. When Mac Conkey agar medium was inoculated with the strain it did not change colour into red. This indicated that strain does not ferment lactose.

Optimization of growth conditions

Table 1. Growth of bacterial strain at 72 hrs in iron added liquid culture media and different temperatures while shaking at 100 rpm.

| Temperature (°C) | 0 | 5 | 15 | 25 | 30 | 35 | 40 | 45 | 55 | 65 |
|------------------|---|-------|-------|-------|-------|-------|-------|-------|-------|----|
| OD (600nm) | 0 | 0.026 | 0.569 | 0.953 | 1.122 | 1.297 | 0.988 | 0.674 | 0.192 | 0 |

Isolated bacterial strain was grown at varying temperatures, ranging from 5 to 55°C in liquid LB media. The temperature range for growth of the isolated strain was 25 to 40°C. This bacterial strain grows at 35°C but no growth at 0 and 65°C (Table 1). This bacterial strain grew well at temperatures between 25–40°C and did not grow at or below 0°C. At 55°C less growth (OD600 nm 0.192) was observed. Optimum growth of the bacteria growing in the iron added liquid media, was observed at 35°C. When different concentrations of NaCl were added to the iron added liquid growth media, the growth (OD at 600nm) of the strain was decreased from 1.264 to 0.453 at 42 h in the liquid media with NaCl concentration was increased from 0 g.L⁻¹ to 10 g.L⁻¹. Beyond 10 g.L⁻¹ concentration of NaCl, the growth of the strain decreased. In the presence of 25 g.L⁻¹ NaCl, no growth (OD 600nm) was observed (Table 2). This bacterial strain grew well at pH values ranging between 5.0 and 10.0 but it did not grow at pH values less than 5.0 and above 10.0. Optimum growth (OD 600nm= 1.383) was obtained at pH 7.0 (Table 3).

Table 2. Growth of bacterial strain at 72 hrs in iron added liquid culture media containing different concentrations of NaCl and 35°C while shaking at 100 rpm.

| NaCl (g.L ⁻¹) | 0 | 1 | 2 | 4 | 6 | 8 | 10 | 25 | 50 |
|---------------------------|-------|-------|-------|-------|-------|-------|-------|----|----|
| OD (600nm) | 1.264 | 1.036 | 0.956 | 0.854 | 0.825 | 0.694 | 0.453 | 0 | 0 |

Table 3. Growth of bacterial strain at 72 hrs in iron added liquid culture media at 35°C and different pH values while shaking at 100 rpm.

| pH values | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|------------|---|-------|-------|-------|-------|-------|-------|----|----|
| OD (600nm) | 0 | 0.221 | 0.768 | 1.383 | 1.024 | 0.592 | 0.089 | 0 | 0 |

Final confirmation of species of identified strain *Gallionella*

Characteristics of the isolated bacterial strain were compared with other iron bacterial species. If the character of strain is similar to the known species its score would be 1. If the character is not similar and variable, it will not get any score. Total score was counted, divided by total characteristics and it was multiplied by 100 and presented as a percentage. Based on these morphological findings and biochemical studies, the isolated strain got the highest score of 95% showing similarities with *Gallionella ferruginea*. The strain showed clear characteristics of *Gallionella ferruginea* than the other suspicious iron bacterial species. As the bacterial strain got the highest score, it was identified as *Gallionella ferruginea*.

Molecular Identification of bacterial isolate

The alignment results using the BLAST algorithm showed that the 16S rRNA sequence was from a bacterium *Gallionella ferruginea*. The most common water complaints are those of red water, laundry spotting, metallic tastes, and staining of plumbing fixtures. These are usually due to the presence of iron above 0.3 mg.L⁻¹. The smells of most of the water of the collector well two is like irony. It may be due to the corrosion of iron pipes collecting water in the Vallipuram area. The shortage of iron causes a disease called “anaemia” and prolonged consumption of drinking water with high concentration of iron may be lead to liver disease called as haemosiderosis. Industrialization without proper waste management system and excessive applications of fertilizers and pesticides in agriculture are the two principle reasons of water pollution. Similar experiments

have been performed worldwide to determine the water quality of diverse water sources (Health Canada 2007, Kalwela & Savale 2012, Kapilan 2015, Perera *et al.* 2012, Tiimub *et al.* 2012).

Table 4. Brief summary of the bio chemical tests done on the water samples collected at different sources in the Vallipuram area.

| Bio Chemical Tests | Sample 01 | Sample 02 | Sample 03 | Sample 04 | Sample 05 | Sample 06 |
|------------------------|---------------|-----------|-----------|---------------|---------------|-----------|
| Catalase | Negative | Negative | Negative | Negative | Negative | Negative |
| Oxidase | Negative | Negative | Negative | Negative | Negative | Negative |
| Indol (layer Pink) | Possitive | Possitive | Possitive | Possitive | Possitive | Possitive |
| Methyl red | Negative | Negative | Negative | Possitive | Possitive | Possitive |
| Voges-Proskauer | Negative | Negative | Negative | Negative | Negative | Negative |
| Citrate | Possitive | Negative | Negative | Possitive | Possitive | Negative |
| Triple Sugar-Iron Agar | Acid/Alkaline | Negative | Negative | Acid/Alkaline | Acid/Alkaline | Negative |
| Glucose | Possitive | Negative | Possitive | Possitive | Possitive | Possitive |
| Lactose | Possitive | Negative | Negative | Possitive | Possitive | Negative |
| Sucrose | Possitive | Negative | Possitive | Possitive | Possitive | Negative |
| Maltose | Possitive | Negative | Negative | Possitive | Possitive | Negative |
| Mannitol | Possitive | Possitive | Negative | Possitive | Possitive | Possitive |

Note: Sample 01 → Collector Well - 04 Water Sample; Sample 02 → Sum well Water Sample
 Sample 03 → Collector Well - 02 Water Sample; Sample 04 → Collector Well - 03 Sludge; Sample 05
 → Collector Well - 03 Water Sample; Sample 06 → Collector Well - 01 Water Sample

Gallionella ferruginea is an iron-oxidizing chemolithotrophic bacteria that has been found in ground water. These bacteria show a crucial role in fixing and oxidizing iron (Fe). It is a genus of stalked, ribbon-like bacteria which employ iron in their metabolism, and cause staining, plugging and odour issues in well water system (Anderson & Pedersen 2003). They cause dangerous problems in well water systems. Because it is challenging to eradicate *Gallionella ferruginea* once they have entered well systems, prevention is the best safeguard. This research is concerned primarily with the behaviour of iron in solution. Iron occurs in two oxidation states, the divalent or ferrous form and the trivalent or ferric form. Iron in aqueous solution is subject to hydrolysis. The iron hydroxides formed in these reactions. The ferric form, have very low solubility (Aiyesanmi *et al.* 2008). The retention of iron in solution is consequently affected by the pH of the solution. In most natural waters, the pH is not low enough to prevent hydroxides from forming, and under oxidizing conditions, practically all the iron is precipitated as ferric hydroxide (Perera *et al.* 2012). Irons have a tendency to form complex ions with inorganic and organic materials in solution state. These ions may be considerably more stable than the noncomplex ion and more may remain in solution that might spoil the quality of the drinking water. The primary source of iron is the water bearing strata (Maddison & Gagnon 1999, Perera *et al.* 2012). Corrosion of iron pipes in a water distribution system can cause three different types of problems: 1. The pipe mass is lost through oxidization to soluble iron species or iron-bearing scale. 2. The scale can accumulate as large tubercles that increase head loss and decrease water capacity. 3. There will be a release of soluble or particulate iron corrosion-by products into the good quality water. This will decrease the aesthetic quality of water and often leads to yellowish or reddish water at the tap. Therefore corrosion of iron pipes has become as a serious issue in the water industry.

CONCLUSION

Based on the microscopic, biochemical and culture studies, the isolated strain from the drinking water collector wells at Vallipuram area of the Northern Sri Lankawas identified as *Gallionella ferruginea*. This bacteria was very active in growth at pH 7.0 and temperature 35°C in iron added liquid growth media. Reddish brown slime formation and bad odour of the drinking water from the fresh water collector wells at Vallipuram area could be prevented or minimized by the addition of NaCl at very low concentration.

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REFERENCES

- Ananathanarayan R & Paniker CKJ (1997) In: Paniker CKJ (ed) *Textbook of Microbiology*. Ind com Press, Chennai, pp. 46–49.
- Anderson CR & Pedersen K (2003) *In situ* growth of *Gallionella* biofilms and partitioning of lanthanides and actinides between biological material and ferric oxyhydroxides. *Geobiology* 1(2): 169–178.
- Barrow GI & Feltham RKA (1993) In: Barrow GI & Feltham RKA (eds) *Cowan and Steel's Manual for the identification of medical bacteria*. Cambridge University Press, Great Britain, pp. 51–93. [ISBN 0521 32611 7]
- Chapnick MS, Tillman DH & Bunch BW (1982) Microbial mediated manganese oxidation in fresh water lake. *limnology and Oceanography* 27: 1004–1014.
- Chessbrough M (1984) Medical Laboratory manual for Tropical countries. In: Hessbrough M (ed) *Microbiology*. Cambridge, Great Britain, pp. 225–248. [ISBN 0 8385 3612 3]
- Fisher S (1975) Endospore-forming rods and cocci: Family Bacillaceae. In: Buchanan RE, Gibbons NE, Cowan ST, Holt JG, Liston J, Murray RGE, Niven CF, Ravin AW & Stanier RY (eds) *Bergey's Manual of Determinative Bacteriology*. Waverly Press, USA, pp. 529–550. [ISBN 0 683 01117 08]
- Ghiorse WC (1984) Biology of iron and manganese depositing bacteria. *Annual Review of Microbiology* 38: 515–550.
- Goonetilleke A, Thomas E, Ginn S & Gilbert D (2015) Understanding the role of land use in urban storm water quality management. *Journal of Environmental Management* 74(1): 31–42.
- Halbach M, Koschinsky A & Halbach P (2001) Report on the discovery of *Gallionella ferruginea* from an active hydrothermal field in the deep sea. *Inter Ridge News* 10(1): 18–20.
- Hallbeck L & Pedersen K (1990) Culture parameters regulating stalk formation and growth rate of *Gallionella ferruginea*. *Journal of General Microbiology* 136: 1675–1680.
- Hamilton WA (1985) Sulphate-Reducing Bacteria and Anaerobic Corrosion. *Annual Review of Microbiology* 39: 195.
- Hasselbarth U & Ludemann D (1972) Biological incrustation of wells due to mass development of iron and manganese bacteria. *Water treatment and Examination* 21: 20–29.
- Health Canada (2007) Total Fecal & *E. coli* Bacteria in Ground water. In: *Water Stewardship Information series*. Available from: http://www.env.gov.bc.ca/wsd/plan_protect_sustain/groundwater/library/groundfact_sheets/pdfs/coliform%28020715%29_fin2.pdf. (accessed: 08 Aug. 2015).
- Iversen A (2001) Microbiologically influenced corrosion on stainless steels in waste water treatment plants: Part 1. *British Corrosion Journal* 36(4): 277–283.
- Jpirgensen BB (1989) Biogeochemistry of chemoautotrophic bacteria. In: Schlegel HG & Bowien B (eds) *Autotrophic Bacteria*. Springer-Verlag, Berlin, pp. 193–218.
- Kaiser G (2001) Prokaryotic cell structure: The Gram Positive cell wall. In: Kaiser G (ed) *Doc Kaiser's Microbiology*. BC Publishing Company, India, pp. 160–184. [ISBN 0 561 30245 76]
- Kapilan R & Arasaratnam V (2010) A novel *Bacillus pumilus* strain for alkaline xylanase production at and above 40°C. *Ceylon Journal of Science (Bio. Sci.)* 39(1): 61–70.
- Kapilan R (2015) Determination of drinking quality of water near the coastal areas of Jaffna lagoon. *Journal of progressive research in Biology* 2(1): 32–36.
- Meisler, Dissanayake CB & Senaratne A (1977) Paleogeographical reconstruction of Jaffna peninsula. *Journal of the Geological Society of India* 23: 369–377.
- Sayanthan A, Saravanan S & Kapilan R (2015) Physico-chemical analysis of drinking water from the collector wells in Vallipuram costal area of the Jaffna peninsula, Sri Lanka. *International Journal of Research and Innovations in Earth Science* 2(6): 168–176.
- Senaratne A & Dissanayake CB (1982) Palaeogeographic Reconstruction of Jaffna Peninsula, Sri Lanka. *Journal Geological Society of India* 23: 545–550.

- Simon AN, Salunkhe I, Arvindkumar S, Goodluck HA, Wafaa SM, Mourad-Agha & Hanna CP (2013) Control of Iron Metabolism in Bacteria. In: Banci L (ed) *Metallomics and the Cell. Series Metal Ions in Life Sciences*. Springer, pp. 203–239. [ISBN 978 94 007 5560 4]
- Staley JT, Bryant M, Pfennig N & Holt JG (1989) *Bergey's Manual of Systematic Bacteriology, Vol. 3*. Williams and Wilkins Company, Baltimore, pp. 1601–2298.
- Theivendrarajah K (1990) *Microbiology Laboratory Manual: Department of Botany, University of Jaffna*. University Publication, Jaffna, pp. 1–33.
- Wijesekera J, Senaratne A & Gunatilake AAJK (2012) Land gain in northern Sri Lanka, Proceed. Auto Carto 2012 - Columbus, Ohio, USA - September, pp. 16–18.