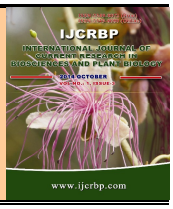
EXCELLENT PUBLISHERS

International Journal of Current Research in Biosciences and Plant Biology

ISSN: 2349-8080 Volume 1 Number 3 (October-2014) pp. 75-79

www.ijcrbp.com



Short Communication

Isolation of Iron Bacterium, *Gallionella ferruginea* from Soil Samples Adherent to Rusted Iron Pipes and its Role in Coliform and Dye Reduction

Tariq Ahmad Lone[1]* and Reyaz Ahmad Lone[2*]

[1]Department of Microbiology Sree Amman Arts and Science College, Erode-638 102, Tamil Nadu, India
[2]Department of Biotechnology, Periyar University Salem-636 011, Tamil Nadu, India

**Corresponding author.*

Abstract

Iron bacteria were isolated from the soil sample collected at rusted iron pipes in industrial area of Erode, Tamilnadu, and identified. The cells were Gram negative short rods with strict anaerobic. The biochemical characterization showed the positive interpretation for indole, methyl red and citrate while Voges-Proskaeur, catalase and oxidase tests showed negative. These fermented sugars, glucose, lactose, sucrose, maltose and mannitol, hydrolyzed the starch and reduced nitrate. The bacteria were capable of fermenting glucose with the production of acid in both aerobic and anaerobic condition, thus confirmed as *Gallionella ferruginea*. The fecal coliforms from the aquarium waste water declined when iron bacteria was supplied and incubated for 48 h. The analysis of dye reduction capability by the *G. ferruginea* showed the maximum reduction of the yellow colour dye followed by orange, green and violet colour dyes after seven days of incubation.

Keywords

Coliforms
Dye reduction
Gallionella ferruginea
Iron bacteria
Microbial corrosion

Introduction

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). The participation of microorganisms in a corrosion process was ignored in the past but is now acknowledged and remains the focus of present and future research work (Benmoussat and Hadjel, 2005). The presence of biofilm on a metal surface often leads to highly localized changes in the concentration of the electrolyte constituents, pH and oxygen levels (Jan and Iwona, 2004). The reactions energized by nutrients obtained from the surrounding environment (Straub et al., 2001). Bacteria flourish only in the presence of certain substances such as organic food nutrients, e.g. sugar, inorganic salts, sulphates necessary for their growth and multiplication (Wolfgang and Tilman, 2006). Microbiologically influenced corrosion is a process that involves the colonization of various

types of fungi, algae, and aerobic/anaerobic bacteria (Quatrini et al., 2007). When the metal changes from aerobic to an anaerobic state, aerobic bacteria provide food to the aerobic cell structure and the inner areas of the biofilm are often eight to ten cells thick and represent an anaerobic environment with large pH differences existing between differentiating layers (Bernard and Alain, 2006). This evolving environment contributes to the formation and growth of specific types of microbiologically influenced corrosion within a water based metal (Strand et al., 2010).

Microbiologically influenced corrosion is a reoccurring problem that has attacked nearly all common engineering metals and alloys including carbon steel, lined steel, stainless steels, aluminum, copper and high-nickel at varying rates (Valde et al., 2008). The only metal material titanium known to resist both aerobic and anaerobic microbiologically influenced corrosion (Yarza´bal et al., 2004). This special mental will allow growth and attachment of biofilm on the mental surface, but does not allow bio toxic environments to affect the physical integrity surrounding it. Corrosion has never been reported under any biofilm that has established growth on titanium (Charackils and Cooksey, 1983).

Factors and areas that contribute to these liking areas weld metal, metallurgical material along the fusion zone, increased surface areas, possible thin walls, and the heat-affected zones on welds after two years of service (Puyate and Rukesh, 2008). Iron is found in most waters in numerous forms: in true solution, as a colloid, in suspension, or as a complex with other mineral or organic substance, it can impart a bitter taste when present in large amounts, marking the water unpalatable (Weber et al., 2006). The present study was aimed to isolate, identify iron bacteria from soil samples adhering to rusted iron pipes of industrial area in Erode town, Tamil Nadu.

Materials and methods

Isolation and identification of iron bacteria

In order to study the role of iron bacteria and their existence in microbial corrosion it was needed to isolate the bacteria from the literature survey we could find that the most economical losses were cause due to microbial corrosion to the metallic structures in aquatic environment. Soil samples were collected from rusted iron pipes in the industrial area-Thirunagar colony, Erode, Tamilnadu (Emerson and Floyd, 2005). The iron bacteria were isolated from the soil by serial dilution method using pour plate method on the Winogradsky medium and incubated at 37°C for 24 h (Mahbubar et al., 2010).

The colony morphology was noted for the appropriate size, elevation or flat, margin, uniform or wrinkled. Gram staining and motility tests were performed for studying bacterial morphology and motility. The biochemical tests such as indole, methyl red, Voges Proskauer, citrate, triple sugar iron, sugar fermentation tests (glucose, lactose, sucrose, maltose and mannitol), respiratory tests (catalase and oxidase) and amino acid utilization test were performed (Staley et al., 1989). Various cultural tests such as starch hydrolysis test, Hugh and Lifesons test, nitrate reduction and urease test were performed to identify the presence of iron bacteria (Hallbeck and Pedersen 1990; Hedrich et al., 2011).

Coliform Reduction by Iron Bacteria

The aquarium water collected and was subjected to most probable number test to estimate the number of fecal coliforms present in 100 ml of the sample by following the method of Gruett (1993). Lauryl tryptose broth (LTB) was prepared in three double strength test tubes by taking 20ml of LTB medium in each and three single strength test tubes by taking 10ml of LTB medium in each. Durhams tubes were placed in all the test tubes and medium was sterilized and cooled to room temperature. A loop full culture of iron isolated bacteria was inoculated to each of the test tubes and incubated at 37°C for 24 h. The positive result was indicated by the gas formation in Durhams tubes.

A Brilliant green lactose bile broth (BGLB) was prepared, sterilized and cooled to room temperature. A loop full of culture form the positive result shown in LTB test tubes were transferred to BGLB test tubes and incubated at 37°C for 48 h. The result was noted by the gas formation in the Durhams tube. The same procedure was followed with 100ml of aquarium water treated with 50ml of iron isolated bacterial culture and incubated at 37°C for 48 h. After incubation coliform count was noted.

Dye Reduction Test

Dye reduction test was performed with the iron isolated bacterial culture by using 0.1g/100ml concentration of various dyes such as violent, orange, green and yellow dye solutions were prepared. The isolated iron bacteria culture was inoculated into individual dye solution and incubated at 37°C for 7 days at an interval of each day the absorbance values for the dye solution was observed on the Spectrophotometer at 620nm.

Results

Total plate count was retrieved using nutrient agar containing iron drops, following the standard procedure. Serial dilution technique was done and the sulphur reducing bacterial count ranged 18-22 in iron supplemented nutrient agar and incubated anaerobically. Morphological characteristics of sulphur reducing bacteria were found having 0.2-0.4mm size, raised elevation, brown colour and regular margin colonies. Under microscopic examination Gram negative rod shaped bacteria and motility were seen.

After incubation of biochemical tests such as Indole, Methyl Red,Voges Proskauer, Citrate, Sugars viz glucose, lactose, sucrose, maltose, mannitol, Respiratory tests such as Catalase, Oxidase and Amino acid utilization test confirmed the presence of *Gallionella ferruginea* are presented in Table 1. The isolated iron bacteria which has hydrolyzed the starch, urease, reduced nitrate and were capable of fermenting glucose with the production of acid in both aerobic and anaerobic condition which indicated glucose fermentation in the presences or absence of oxygen thus confirming the presence of the *Gallionella ferruginea* as all the characteristics belongs to the same bacteria.

Among the test tubes LTB medium all the double strength test tubes and three of the single strength test tubes were found to be positive. The four of the test tubes with BGLB medium showed positive result. By comparing these values with the MPN chart in Mccardy's table the coliform count was found to be 700. Among the LTB test tubes which used the aquarium water after treating with culture one of the double strength and three of the single strength test tube were found to be positive. The three of the test tubes with BGL medium showed positive result, by comparing the values with Mccardy's table the coliform count to be reduced to 400. The analysis of dye reduction capability by the *Gallionella ferruginea* showed the maximum reduction in the optical densities of the yellow colour dye and followed by the orange, green and violet colour dyes after seven days of incubation (Table 2).

Table 1. Biochemical test of the Iron bacterium, *Gallionella ferruginea*

Name of Test	Result	Name of test	Result
Indole	Positive	Oxidase	Negative
Methyl Red	Positive	Glucose	Fermented
Voges Proskauer	Negative	Lactose	Fermented
Citrate	Positive	Sucrose	Fermented
Triple sugar iron	Acid/ alkaline	Maltose	Fermented
Catalase	Negative	Mannitol	Fermented

Table 2. Dye reduction capability of iron bacterium, *Gallionella ferruginea*

Incubation Period (days)	Colour of the dyes used*			
	Violet	Orange	Green	Yellow
Initial	0.25	0.11	0.16	0.06
1	0.25	0.10	0.16	0.05
2	0.24	0.10	0.14	0.06
3	0.22	0.09	0.13	0.05
4	0.22	0.09	0.12	0.05
5	0.20	0.08	0.12	0.04
6	0.18	0.08	0.11	0.04
7	0.18	0.08	0.10	0.04

*OD values recorded at 620nm.

Winogradsky as a standard method medium containing ferric ammonium citrate used to obtain cultures of heterotrophic iron bacteria like *Liptothrix* and autotrophic species like *Gallionella ferruginea.* The respective result of biochemical tests for the iron bacteria confirmed the presence of *Gallionella ferruginea* in microbial corrosion zones. The colonization involves the formations of Biofilms follow a similar growth process on most materials and consists of the chain of events (Charackils and Cooksey, 1983). The matrix material adhering the microbes to each other and to the metal surface is made up of a highly hydrated anionic polysaccharide polymer (Miller and King, 1975). The adhering material bridges microbial cells together, thus allowing the localized environments and water conditions change constantly, anaerobic

bacteria can survive long exposures to oxygenated environments with little adverse effects (Ghiorse, 1988). Microbiologically influenced corrosion has the ability to induce corrosion anywhere in a metal based system and has favorable liking for weld areas, especially on stainless steels (Iveson, 1966) The previous study summarized the difficult of controlling iron bacteria by the statement iron bacteria are tenacious and continue to grow even after the severest kind of treatment and if relief is to be had it is likely to be temporary (Hedrich et al., 2011).

Recently microbiologically influenced corrosion has been known to affect fire protection systems in various industries thought the county (Strand et al., 2010). While there are many proposed methods to control microbiologically influenced corrosion suggest that mechanically disturbing the biofilm well help retard microbiologically influenced corrosion while also enhancing the penetration and effectiveness of biocides (Adieze et al., 2003). It is extremely difficult for biocides alone to penetrate through all microbial cellular layers (Pryfogle, 2002). Corrosion inhibitors at low populations, therefore proper adjustment of biocides are needed to maintain optimum performance (Wagner et al., 2007). The coliform reduction capacity of iron bacteria was tested by most probable number method and could find appreciable reduction in counts. So it can be used for treating aquarium water. Due to low reduction capacity of iron bacteria, it cannot be used for treating highly polluted water. In order to prove the significance of iron bacteria in bioremediation, the capability of iron bacteria is to reduce dyes. The intensity of orange dye was reduced from 0.4076 to 0.10. Thus it can be used for industrially to reduce the pollution in water bodies. But the reduction was not appreciable for yellow color, so it can be used for selective colors of different dyes.

Conclusion

The study has clearly indicated that there is a substantial body of information available concerning microbial corrosion. The iron bacterium has got the ability to reduce dyes and coliforms. In order to interpret field observations, there is a need to perform more experiments in near natural systems.

References

Adieze, I.E, Nwabueze, R.N., Onyze, G.O.C., 2003. Effect of poultry manure on the microbial utilization of hydrocarbons in oil-polluted soil. Nigerian J. Microbiol. 17(1), 12-16.

Benmoussat, A., Hadjel, M., 2005. Corrosion behavior of low carbon line pipe steel in soil environment. J. Corros. Sci. Eng. 6(9), 1178-1183.

Bernard, M., Alain, P., 2006. Iron-bacterial mediation in Phanerozoic red lime stones: State of the art sedimentary. J. Geol. 185, 147–157.

Charackils, W.G., Cooksey, K.E., 1983. Biofilms and microbial fouling. Adv. Appl. Microbiol. 29, 93-97.

Dexter, S.C., Duquette, D.J., Siebert, O.W., Videla, H.A., 1991. Use and limitations of electrochemical techniques for investigating microbiological corrosion. J. Corros. 47, 308-318.

Emerson, D., Floyd, M.M., 2005. Enrichment and isolation of iron oxidizing bacteria at neutral pH. Meth. Enz. 397, 112–123.

Ghiorse, W.C., 1988. Microbial reduction of manganese and iron. In: Biology of anaerobic microorganisms (Ed.: Zehnder, A.J.B.), John Wiley, New York. pp. 305-331.

Gruett G., 1993. Removing problem iron. Water Techn. 16(3), 48-51.

Hallbeck, L., Pedersen, K., 1990. Culture parameters regulating stalk formation and growth rate of *Gallionella ferruginea*. J. Gen. Microbiol. 136, 1675–1680.

Hedrich, S., Michael, S.M., Johnson, B.D., 2011. The iron-oxidizing proteobacteria. Microbiol. 157, 1551–1564.

Iversen, A., 2001. Microbially influenced corrosion on stainless steels in waste water treatment plants: Part 1. British Corros. J. 36(4), 277-283.

Iveson, W.P., 1966. Direct evidence for the cathodic depolarization theory of bacterial corrosion. J. Sci. 151, 986-988.

Jan, S., Iwona, B.B., 2004. Biocorrosion: towards understanding interactions between biofilms and metals. Curr. Opinion Biotech. 15(3), 181–186.

Mahbur, R.K., Mihir, I.S., Nahmina, B., Mohammad, N.S., Sirajul, H., 2010. Isolation and characterization of bacteria from rusted iron materials. Bangladesh J. Bot. 39(2), 185-191.

Miller, J.D.A., King, R.A., 1975. Biodeterioration of metals in microbial aspects of the deterioration of materials. (Eds: Lovelock, D.W., Gilbert, R.J.). Academic Press, London.

Pryfogle, P.A., 2002. Geothermal biocorrosion. US Dept. Energy Conver. Technol. Bull. Energy. 317, 16-18.

Puyate, Y.T., Rukesh, R.A., 2008. Biocidal efficacy of dissolved ozone, formaldehydes and sodium hypochlorite against total plank tonic microorganisms in produced water. J. Sci. 8(5), 860-865.

Quatrini, R., Valde, S.J., Jedlicki, E., Holmes, D.S., 2007. The use of bioinformatics and genome biology to advance our understanding of bioleaching microorganisms. In: Microbial Processing of Metal Sulfides (Eds.: Donati, E., Sand, W.). Springer, New York. pp. 221–239.

Staley, J.T., Bryant, M., Pfennig, N., Holt, J.G., 1989. Bergey's Manual of Systematic Bacteriology. Vol. 3. Williams and Wilkins Company, Baltimore. pp. 1601-2298.

Straub, K.L., Benz, M., Schink, B., 2001. Iron metabolism in anoxic environments at near neutral pH. FEMS Microb. Ecol. 34, 181–186.

Strand, H., Lapidus, A., Paces, J., Ulbrich, P., Vlcek, C., Paces, V., Haselkorn, R., 2010. Complete genome sequence of the photosynthetic purple non-sulfur bacterium *Rhodobacter capsulatus* SB 1003. J. Bacteriol. 192, 3545–3546.

Valde, S.J., Pedroso, I., Quatrini, R., Dodson, R.J., Tettelin, H., Blake, R., Eisen, J.A., Holmes, D.S. 2008. *Acidithiobacillus ferrooxidans* metabolism: from genome sequence to industrial applications. BMC Genomic. 9, 597.

Wagner, C., Mau, M., Schlomann, M., Heinicke, J., Koch, U., 2007. Characterization of the bacterial flora in mineral waters in upstreaming fluids of deep igneous rock aquifers. J. Geophys. Res. 112(G1), G01003.

Weber, K.A., Achenbach, L.A., Coates, J.D., 2006. Microorganisms pumping iron: anaerobic microbial iron oxidation and reduction. Nat. Rev. Microbiol. 4, 752–764.

Wolfgang, S., Tilman, G., 2006. Extracellular polymeric substances mediate bioleaching or biocorrosion via interfacial processes involving iron (III) ions and acidophilic bacteria. Res. Microbiol. 157(1), 49–56.

Yarza´bal, A., Appia-Ayme, C., Ratouchniak, J., Bonnefoy, V., 2004. Regulation of the expression of the *Acidithiobacillus ferrooxidans* rus operon encoding two cytochromes c, a cytochrome oxidase and rusticyanin. Microbiol. 150, 2113–2123.