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ISOLATION OF CORROSIVE MICROORGANISMS AND IT'S ROLE IN CORROSION INDUCTION AND PREVENTION

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Abstract: Several Bacteria and Fungi isolates were recovered from iron fabrication shop soil materials using modified Barr's media, Nutrient and Potato-dextrose agar media. Five bacteria and seven different fungi isolates were selected after primary screening on the basis of their growth in modified Barr's medium. Out of five bacteria; four belongs to *Bacillus* family and one Bacteria is *Pseudomonas*. Moreover, seven different fungi belong to two from *Aspergillus* family and remaining is *Fusarium, Trichoderma, Verticillium, Cladosporium,* respectively. The organisms showed better growth in presence of iron salts like ferrous sulfate, ferric sulfate and ammonium ferric citrate. Five isolates were found to transform ferrous (Fe^{2+}) to ferric (Fe^{3+}) of which *Bacillus subtilis* was most efficient. A good number of bacteria were associated with oxidation of iron.

Keywords- Corrosion, Microbiologically-influenced corrosion, Barr's Medium, SRB medium.

INTRODUCTION

Corrosion is a natural process, which converts a refined metal to its oxide or hydroxide or another compound which is a more stable form, or can be generally defined as the gradual destruction of materials by microorganisms or by chemicals and electrochemical reactions with their environment. Microbiologically-influenced corrosion (MIC) is defined as the deterioration of metals as a result of metabolic activities of microbes. The biological harmful activities modify local chemistry and render it more corrosive to the metal. The aerobic iron and manganese bacteria are mainly responsible about the accelerated pitting attacks of stainless steel, however the anaerobic sulfate reducing bacteria (SRB) are responsible for most highly corrosion damages to offshore steel structures. Stainless and carbon steel tanks, pipelines, heat exchangers, fuel storage tanks are mainly affected by MIC. On the other hand, other beneficial microorganisms play a major role for protecting these surfaces from corrosion via different mechanisms including biofilms formation. The aim of the present study is to present the role of microorganisms on the induction and prevention of corrosion. This includes corrosion inhibition mechanisms employing beneficial microorganisms with special reference to microbial biofilms to avoid the dramatic economic loss due to corrosion. On the contrary, different types of harmful microorganisms included in corrosion which of them is sulfate reducing bacteria (SRB). (Rawia Mansour, 2016)

Corrosion as being one of the most serious problems in our society is resulting into losses each year in hundreds of billions of rupees. Some major losses due to corrosion are enlisted below:

(i) It damages industrial machines and unpredictable machinery failure, which could lead to loss of life.
(ii) It damages metallic equipment such as boiler tubes in thermal power plants.
(iii) It reduces the overall value of that product and wastes the valuable resources.
(iv) Some metallic properties such as conductivity, ductility, malleability, luster etc. are lost due to corrosion.
(v) About 20% of the total production of iron is wasted annually every year due to corrosion.
(vi) It also contaminates portable water.

MATERIALS AND METHOD

The materials and methods used during this study are elaborated under the following heads.

1. COLLECTION OF RUSTING SOIL SAMPLE:

The rusting soil samples were collected from fabrication shop where continuous contact of soil with metal occurs. These samples were collected from five-centimeter surface layer which contain metal powders and different type of living microorganisms.

2. ISOLATION OF MICROORGANISM:

The rusting soil microorganisms were isolated by serial dilution and platting technique. One gram of soil from sample were separately suspended in 10 ml of distilled water and mixed well for 15 minutes and vortexed. Each suspension was serially diluted from 10^{-1} to 10^{-6}. (Blaize) 0.1ml each was pipette out onto the prepared Barr's medium plates (Composition- K_2HPO_4 0.05 g, NH_4Cl 0.1 g, $CaSO_4$ 0.2 g, $MgSO_4.7H_2O$ 0.2 g, Sodium lactate 0.7 g, $Fe(NH_4)_2(SO_4)_2$ 0.05 g, Distilled water 100.0 ml) and incubated at 37°C for three days. (Ghazy, 2011) (K. R. BUTLIN, 1948)

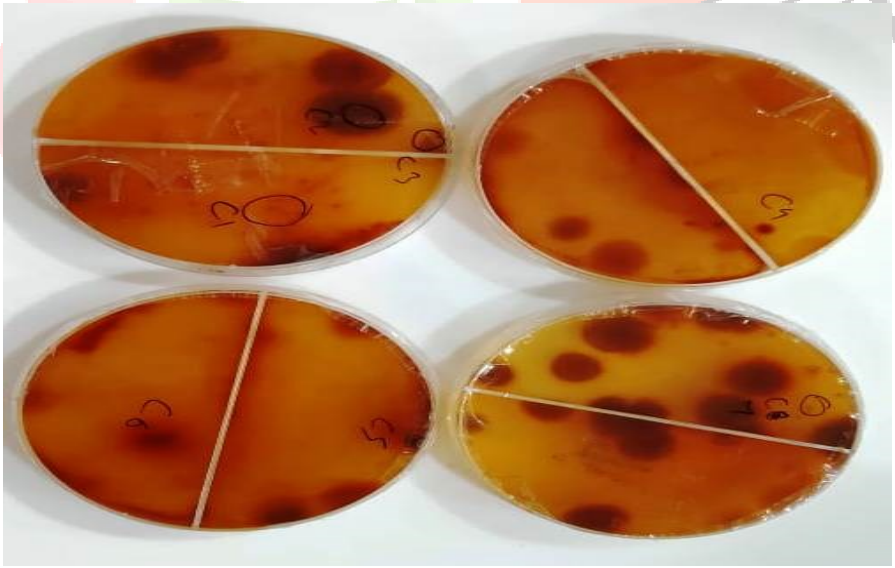


Fig.1. Barrs media plates

3. ISOLATION OF PURE CULTURE BACTERIA AND FUNGI

After 24 hrs. of incubation, for Bacteria, by using streak plate technique culture streak on nutrient agar medium (NAM) plate and for Fungi, by using wire-loop culture streak on Potato-dextrose agar (PDA) slant as well as plate and incubated at 37°C for 24 hours.

4. TUBE PREPARATION WITH ADDITION OF BARR'S MEDIUM, CULTURE AND NAIL (IRON)

To check the activity of isolated microorganism with the Iron, we prepared different volumes of cultured (Bacterial and Fungal) Eppendorf tubes with added Barr's medium and Nail (Iron) and incubated all at 37°C for 7 to 14 days. (Rawat, 2016)

RESULTS AND DISCUSSION

1. IDENTIFICATION OF ISOLATED BACTERIA

Isolated fungi were belonging to the Bacillus and Pseudomonas family which were identified by performing the characterizations and confirmational test. (HEMRAJ, 2013)

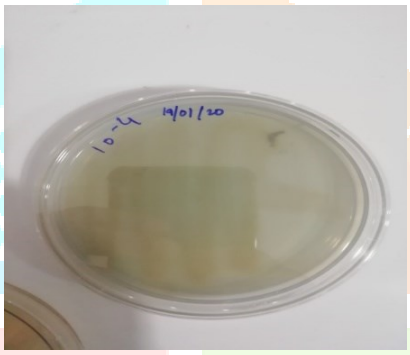


Fig.2.*Bacillus*

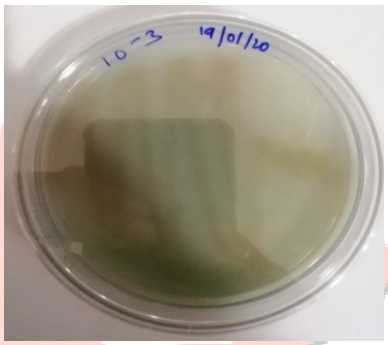
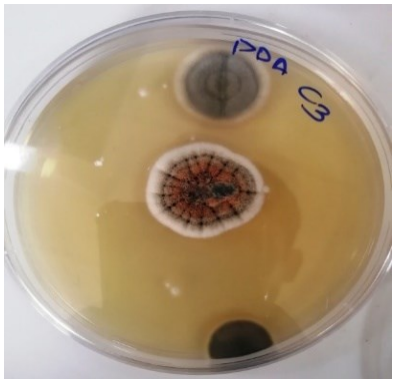


Fig.3.*Pseudomonas*

2. IDENTIFICATION OF ISOLATED FUNGI

Isolated fungi were belonging to the *Fusarium, Aspergillus, Trichoderma, Verticillium, Cladosporium* which were identified by performing the characterizations and confirmational test. Below diagram shows the shape, structure and colour.

Fig.4.Fungi slants



Microscopic view

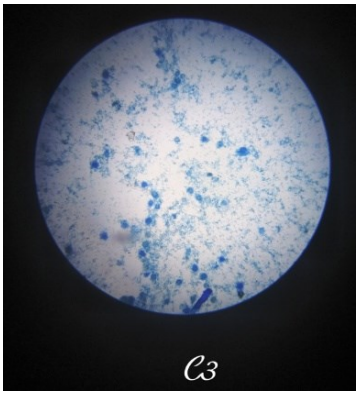


Fig.5. *Fusarium*

Microscopic view

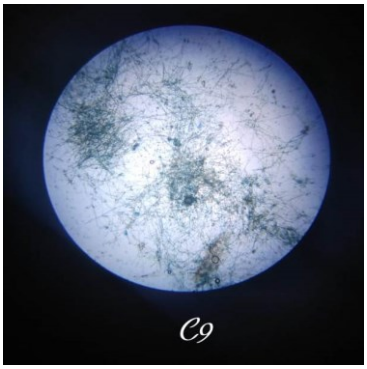
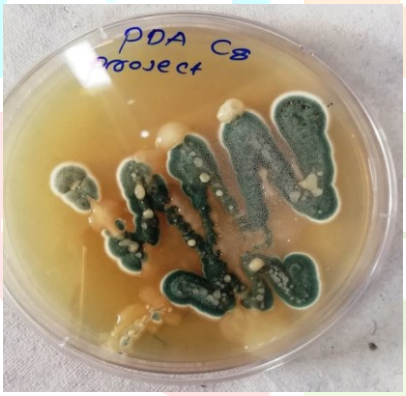


Fig.6. *Aspergillus*



Microscopic view

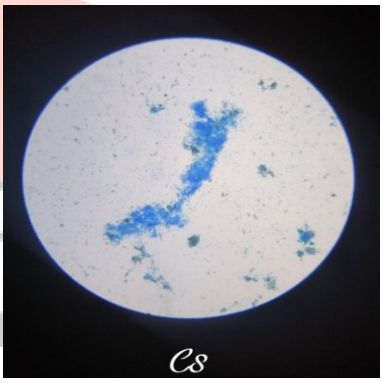


Fig.7. *Aspergillus*

3. BIOCHEMICAL TEST FOR BACTERIA

A. INDOLE TEST

Red colored ring formed on the surface of tube in *Bacillus*, test is Positive.

No ring formation occurred on the surface of tube in Pseudomonas, test is Negative

Fig.8.

B. CITRATE TEST

Citrate test is Positive for Both *Bacillus* and *Pseudomonas*, Colour Changes from Green to Blue

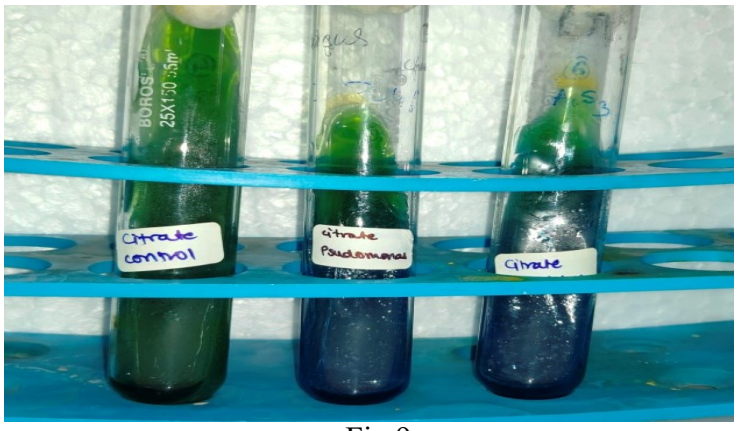


Fig.9.

C. MR-VP TEST

MR test is Positive for *Bacillus* and Negative for *Pseudomonas*.

VP test is Negative for Both

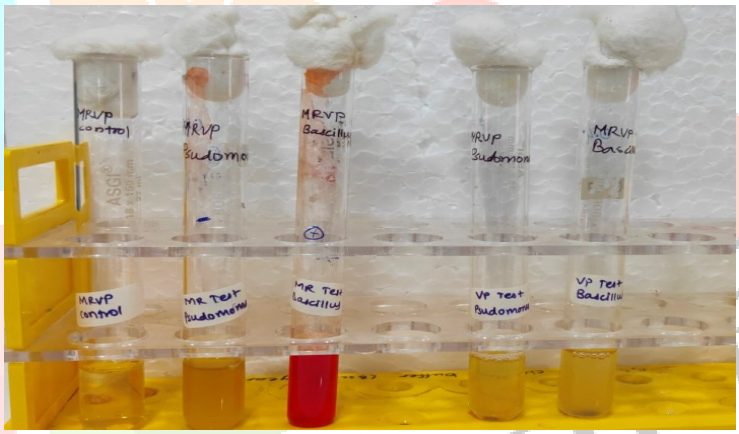


Fig.10.

4. BIOCHEMICAL TEST FOR FUNGI

A. STARCH HYDROLYSIS TEST

Starch test is Positive therefore, all fungal cultures are amylase Producing.

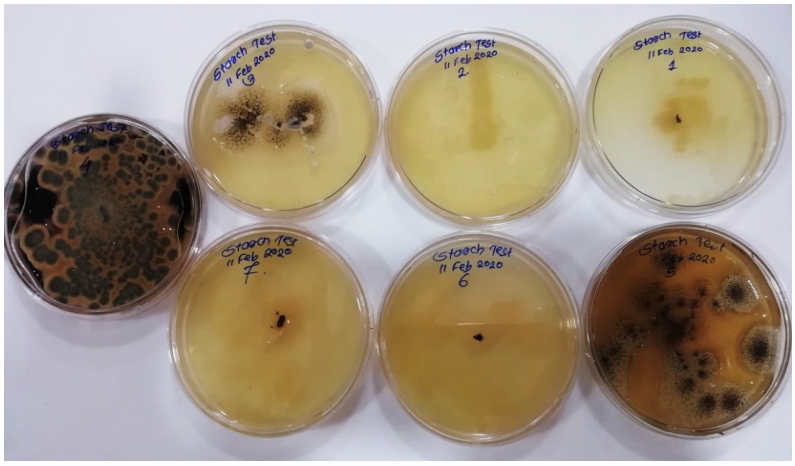


Fig.11.

B. CELLULOSE HYDROLYSIS TEST

Test is Negative there is no clear zone around the fungal growth.

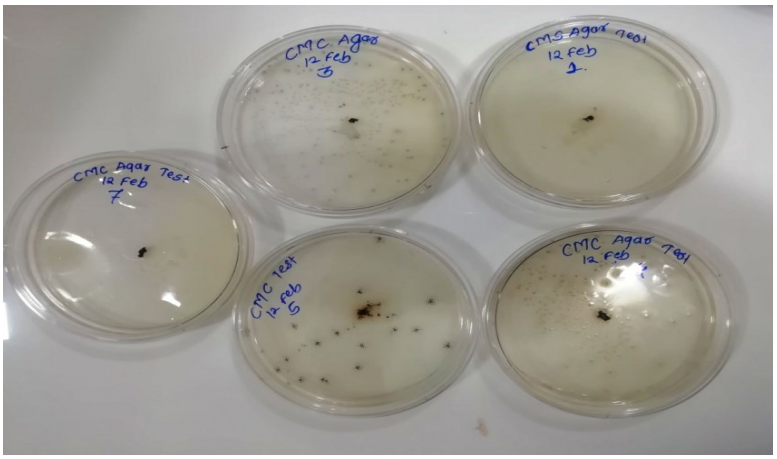


Fig.12.

C. SOLUBILIZATION INDEX TEST

A clear zone around a growing colony indicated phosphate solubilization.

Fig.13.

5. ACTIVITY OF MICROORGANISM IN CONTACT WITH NAIL(IRON)-

Prepared tube containing isolated culture, Barr's medium and Nail produced a rusting on the nails after incubation. Bacterial culture specially in Bacillus tube rusting is more as compared with other culture. Biofilm formation is seen around nail in 2 to 3 days, after 4th day biofilm started to degrade and then color of culture changes dark orange. In the bottom of tube rusted residues of nail increases day by day.

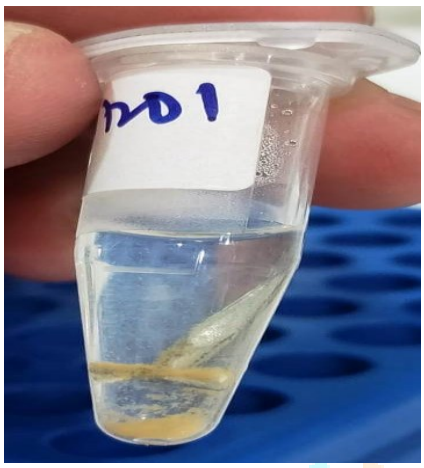


Fig.14. Control (Barr's medium + Nail)

Fig.15. Biofilm formation around Nail

Fig.16. SRB medium+ Nail + *Bacillus* Culture

Fig.17. Rusting on nail occurs with growth of *Bacillus*

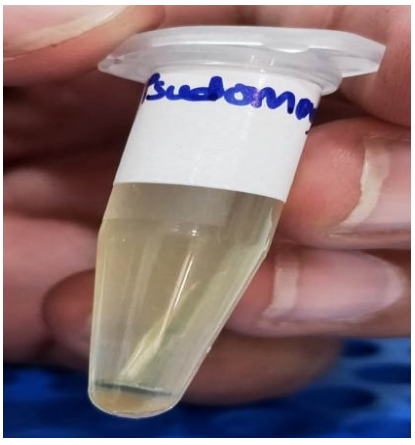


Fig.18. SRB medium+ Nail + *Pseudomonas* culture

Fig.19. Rusting on nail occurs with growth of *Pseudomonas*

DISCUSSION

This is the first to detect and isolate pure culture of corrosive microorganisms found in the iron containing soil by using Barr's medium. The newly isolated bacteria and fungi enhanced the iron corrosion while incubated with it. Further study on corrosion inducing microorganism will shed light on how rigorously corrosion happens? Whenever bacterial or fungal culture changes. The Plant based; study also light up the possibility of milestone.

CONCLUSION

Introducing the **Bacteria and Fungi** (which had isolated from Rusting Soil) into the tube containing Barr's medium and Nail (Iron) produces Rusting (Corrosion) on Nail (Iron) after incubation of 7 to 14 days.

Therefore, it was concluded that introducing microorganisms are corrosion inducing microorganisms.

Microorganisms may be responsible for corrosion along with Chemical and Physical Activity.

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