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Underground Corrosion by Microorganisms–Part-III Role of Soil Inhabiting Actinomycetes

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ABSTRACT

Certain strains of soil inhabiting actinomycetes were found to substantially corrode aluminium alloy (54-S) which has been found to be more resistant to bacterial or fungal corrosion in our earlier studies. These strains did not produce any corrosion on the mild steel and galvanised iron panels which were heavily corroded by bacteria and fungi. The corrosive isolates have been partially characterised after their isolation and purification. The extent of corrosion caused by each strain has been determined.

INTRODUCTION

Actinomycetes are known to be important constituents of soil microflora[1] though their role as biodeteriogens is not very well known. This may be due to their difficult detection, isolation and characterisation processes. However, instances of deterioration caused by this group of organisms, though scanty, are not altogether lacking. Almost all types of materials undergo deterioration by these organisms. Vulcanised rubbers[2], PVC sheetings, pipes, wrappings and coatings[3], cellulose and lignocellulose[4]and keratinous[5] materials have been reported to be biodegradable through these organisms. However, no record is available regarding their effect on metals. The Defence Materials & Stores Research & Development Establishment (DMSRDE), Kanpur undertook these studies as as part of studies on the microbial corrosion of metals. Metal panels were buried underground for 18 months in seven geoclimatic regions of India. Isolation of microorganisms was carried out from the soil adhering to the corroded panels of mild steel, galvanised iron and aluminium

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alloy (54-S). Alongwith other microbes 16 strains of actinomycetes were also isolated. These were screened for their corrosiveness to experimental metal panels in the laboratory. Out of 16 isolates five were found to cause corrosion only to aluminium alloy panels. The corrosion causing ability of each of the isolates was determined and these were partially characterised.

2. MATERIALS AND METHODS

2.1 Isolation, Purification and Characterisation

Stock soil suspension prepared by suspending one gram of soil in 100 ml. of sterile water were used for isolation from soils of each location. Serial dilutions upto 10^{-6} were plated out on Malt Extract Yeast Extract Agar media[6]. The incubations were done at 32° ± 2°C for 14 days after which the colonies of actinomycetes were picked up. Two methods were used for preliminary characterisation of the isolates. The initial grouping was done on the basis on optimum growth pH. The organisms were segregated according to three pH ranges. Acidophils at pH 4.0., Neutrophils around pH 6.5 and Basophils at pH 9.0. The second crieteria for characterisation was media pigmentation and spore chain morphology of the aerial hyphae[7]. For pigmentation studies Peptone Iron Agar[8] was used as a specialised media. Colony colouration was taken as an additional factor. Further characterisation was not done and all the 16 strains were used in the laboratory exposures of metal panels.

2.2 Preparation of the Metal Panels

Panels 50 x 50 mm in size were prepared from the three experimental metals viz. mild steel, galvanised iron and aluminium alloy (54-S). After identification marking the panels were cleaned, degreased and polished as per standard[9] techniques. Each panel was weighed and transferred to clean dry desiccator containing calcium chloride to prevent moisture contact.

2.3 Corrosivity Determination of the Isolates

The corrosiveness of each strain to the experimental metal panels was determined by the loss in weights of the panels exposed to isolates vis a vis the loss suffered by the uninoculated control panels. An average of four replicates was taken for calculating the weight loss.

2.3.1 Inoculation and Incubation

The panels were inoculated after dipping in ethyl alcohol and flame sterilisation. Contact impressions[10] were obtained on the panels by putting them for 24 hours on 2 weeks old colonies of respective strains. These were then transferred to petridishes with sterile glass beeds and mineral salts broth[10]. Incubation was done for 6 weeks at 32° ± 2°C.

2.3.2 Weight Loss Estimation

After the incubation the experimental panels were washed in running water by scrubbing with a brush, derusted by the prescribed procedure[9] dried and weighed.

The weight losses registered by the inoculated and uninoculated (control) panels were calculated.

3. RESULTS AND DISCUSSION

Out of the 16 isolates of actinomycetes investigated by us only 5 strains viz. strain Nos. 3 and 4 from Mysore, No. 6 from Hyderabad No. 10 from Cochin and No. 11 from Kanpur soils were found appreciably corrosive to aluminium alloy. However, none of these strains induced any corrosion on mild steel and galvanised iron panels (Table 1). It may be observed that the remaining 11 strains were not corrosive to any metal. Even in the case of aluminium alloy panels, the extent of corrosion caused varied from strain to strain. Normally most actinomycetes behave as neutrophils having optimum growth near neutrality[11] pH though their growth range varies between pH 4.0 to 9.0.

It is quite interesting to note that the heaviest metal loss was produced by basophils whose optimum growth was around pH 9.0 and above. Two of these strains were from Mysore while the third originated from Cochin soil (Table 2). The only acidophil, isolated from Kanpur soil, was the least corrosive. The neutrophil strain from Hyderabad was also mildly corrosive. All the five strains were aerobic, gram + ,

Table 1. Amount of corrosion caused by different isolates of actinomycetes on the three experimental metals

Strain number	Location of the soils	Wt. loss in mg due to corrosive actinomycetes on the three experimental metals		
		Mild steel	Galvanised iron	Aluminium alloy
	Mysore	2275	1226	21
2.	"	2663	828	46
3.		2712	1413	300
4		2727	1654	314
5.		2638	862	32
6.	Hyderabad	3046	1229	162
7.		2635	821	22
8.		2256	1041	59
9.	Delhi	2448	170	90
10.	Cochin	2208	1168	394
11.	Kanpur	3027	1290	110
12.	Tezpur	1597	661	47
13.	Nagpur	2907	1367	30
14	"	2308	1275	53
15.		1550	1013	5
16		3402	1838	19
	Control	3695	1503	16

Table 2. Partial characterisation of corrosion causing isolated strains of actinomycetes

Sl. No	Location of isolate	Strain number	Optimum growth Temp. (°C)	pH	Growth characters Peptone iron agar		Spore chain and colony characters	Gram	Odour	Remark
	Mysore	3	32 ±2	9.0	Black pigmentation	No pigment	Spore chain coiled and bunched yellowish brown colonies	+	Non specific	Melanin formed
2.	Mysore	4	32 ± 2	9.0	No change in colour	No pigment	Spiral spore chains small of white colonies	+	No detection	
3.	Hyderabad	6	32 ± 2	6.5	No change in colour	No pigment	Short fragmented spore chains, light cream colonies	+	Not clear	
4.	Cochin	10	32 ± 2	9.0	Black pigment in media	No pigment released	Spore chains long and flexous greyish colonies	+	Non specific	Melanin formed
5.	Kanpur	11	32 ± 2	4.0	No change in media pigment	No release of pigment	Coiled spore chains with white compact colonies	+	No odour	

preferred an optimum growth temperature of 32°C and did not produce any pigment on malt extract agar media. Of the three basophil strains which showed heavy corrosion, two strains, No. 3 from Mysore and No. 10 from Cochin produced black pigmentation on Peptone iron agar media indicating melanin production[11]. Similarly the morphological characters indicated by colony colouration, and formation of spore chains of the aerial hyphae are also varying considerably for all the five corrosive strains. It may therefore, be concluded that none of these strains resemble in their basic characters as well as corrosivity rate. These strains show the characters of genus *Streptomyces*[11] but in the absence of any further characterisation, these could not be classified as such and we had to leave it at the order level (Actinomycetales) as a broader classification for these strains. The significant factor of the studies is the observation that these strains are corrosive only to aluminium alloy and not to the other metals viz. galvanised iron and mild steel. This also negates the belief that aluminium alloys are safer and better metals for use in the underground structures as compared to the two metals mentioned above.

4. CONCLUSION

Aluminium alloy (54-S) has been found to be corroded by five strains of actinomycetes isolated from corroded metal panels buried in different parts of India.

These strains however, do not corrode galvanised iron and mild steel panels which are otherwise corroded by bacteria and fungi. The basophillic strains of actinomycetes are more corrosive then the acidophilic or neutrophilic strains.

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REFERENCES

1 Fermor, T.R. & Eggins, H.O.W., *Int. Biodeterioration Bulletin,* **16** (1980), 95-101.

2. Cudnell, A.M. & Mulcock, A.P., *Int. Biodeterioration Bulletin,* **8** (1972), 119-125.

3. Pankhurst, E.S., Nicholle, H.G.M. & Allan, K., Biodeterioration of Pipe Wrappings and Coatings – Microbial Aspects of the Deterioration of Materials, R.J. Gilbert and D.W. Lovelock (Edrs), (Academic Press, London), 1975, pp. 135-151.

4 Khan, M.R., Williams, S.T. & Saha, M.L., *British Journal of Jute Fibre Research,* **3** (1978), 35-42.

5 Novel, J.J. & Nickerson. W.J., Exocellular Keratinase from a *Streptomyces sp.,* Bacteriological Proceedings, 1956, pp. 125-126.

6. Shirling, E.B. & Gottliels, D., *International Journal of Systematics,* **16** (1966), 313-340.

7. Williams, S.T., Goodfellow, M., Alderson, G., Wellington, E.M.H., Sneath, P.H.A. & Sackin, M.I., *Journal of General Microbiology,* **129** (1983), 743-1813.

8 DIFCO Manual, IX Edition, (Difco Laboratories Inc., Detroit), 1977, p. 18i.

9 Code of Procedure for Conducting Studies on Underground and Subsoil Corrosion of Metals – Recommendation of the Corrosion Research Committee, CSIR, 1975.

10. Stranger, M. & Johannson, Fungal Corrosion of the Steel Interiors of a Ships Hold, (Central Institute of Industrial Research, Oslo, Norway), 1980.

1 Williams, S.T., *International Biodeterioration Bulletin,* **21** (1985), 207-209.