*International Journal of Research Studies in Biosciences (IJRSB)*
*Volume 3, Issue 1, January 2015, PP 164-168*
*ISSN 2349-0357 (Print) & ISSN 2349-0365 (Online)*
*www.arcjournals.org*

# Isolation of Microorganisms from Antarctic Soils and Their Use as Possible Corrosion Inhibitors

**Ignatova-Ivanova TS, R. Ivanov, N. Chipev**

Department of Biology
Shumen University
BG – 9700 Shumen, Bulgaria
*radi_cvet@abv.bg*

**Abstract:** *In this study microorganisms from Antarctic soils were isolated using different selective nutrient media. The soils were collected from different habitats of Livingston Island. The microorganisms were isolated and purified to pure culture. The isolated microorganisms were micro-morphologically and macro-morphologically characterized. The results obtained suggested that despite the extreme Antarctic environment the diversity of soil microorganisms was considerably high. Some of the isolated microorganisms were able to protect corrosion. The effect of the studied microorganisms on the corrosive stability of steel samples was studied by micrometer method. The corrosive activity was confirmed by microscopic pictures of the treated steel samples.*

**Keywords:** *Antarctic, microorganisms, corrosion inhibitors*

High latitude ecosystems are typically thought of asextreme environments containing a limited diversity of plants and animals. There is increasing evidence however, that such a view is not correct at the microbiological level, and that the polar regions contain diverse microbes and microbial habitats [1,2, 3,4,5,6,7,8,9].

Antarctica provides habitats for different microbial communities. The communities are dominated by cold-tolerant (but not psychrophilic) prokaryotes that in turn provide local refugia for the growth and development of more complex organisms including eukaryotic microalgae and micro-invertebrates. These consortia offer in sightsinto how diverse life forms may have persisted and evolved during global episodes of extreme cold [3,10,11].

The purpose of this study was to investigate microbial diversity in Antarctic soils using various selective nutrient media and to try to test the ability of some of the isolated microorganisms to protect metal corrosion.

## 1. MATERIALS AND METHODS

### 1.1. Soil Samples

*1.1.1. Sampling Sites*

The soil samples were collected during the austral summer of 2013 from ice-free areas of Livingston Island at a depth of 0–5 cm. The sites were chosen to represent different habitat characteristics and noted as follows: **S1 (**Argentinian Bay); **S2** (lake bank); **S3** (South Bay); **S4** (bare soil on rocky hill); **S5** (Beach) and **S6** (soil under mosses).

*1.1.2. Soil Analysis*

Water and humus content as well pH of soil samples was determined as it was described in [1].

*1.1.3. Cell Suspensions*

Cell suspensions were prepared from 5 g soil samples suspended in 45 ml sterile 0.9% NaCl (Sigma- Aldrich), described in[1].

*1.1.4. Bacterial Enumeration*

The cultural method was chosen to plate decimal dilution series ($10^{-1}$–$10^{-5}$) of soil suspensions and to enumerate copiotrophic bacteria and actinomycetes. The inoculated agar plates were incubated in dark at $4^{o}$C for 8 days.

### 1.2. Media

- **Media with yeast and malt extracts (M 2) in content** g/l: glucose - 4; yeast extract - 4; malt extract -10; agar-agar – 20. pH 7,3.
- **Media oat agar (M 3) in content** g/l: oat meal – 20; agar-agar – 18; $FeSO_4.7H_2O$ – 0,1; $MnCl_2.4H_2O$ – 0,1; $ZnSO_4.7H_2O$ – 0,1. pH – 7,2.
- **Media starch agar (M 4) in content** g/l: starch – 10; $K_2HPO_4$ – 1; $MgSO_4.7H_2O$ – 1; .NaCl – 1; $(NH_4)_2SO_4$ – 2; $CaCO_3$ – 2, agar-agar – 20. pH – 7,0 -7,4.
- **Media glycerol-asparagines agar (M 5) in content** g/l: L asparagines– 1; glycerol – 10; $K_2HPO_4$ – 1; agar-agar – 20. pH 7,0 – 7,4.
- **Media Gauze agar in content:** starch – 20; $KNO_3$ – 1; $K_2HPO_4$ – 0,5; $MgSO_4.7H_2O$ – 0,5; NaCl – 0,5; $FeSO_4.7H_2O$ – 0,01; agar-agar – 30. pH 7,2 – 7,4.
- **Media starch-ammonia agar SSA in content** g/l: starch – 10; $(NH_4)_2SO_4$ – 2; $K_2HPO_4$ – 1; $MgSO_4.7H_2O$ – 1; NaCl – 1; $CaCO_3$ – 3, agar-agar – 20. pH – 7,0.

The media were sterilized by autoclaving at $121^0$C for 20 min.

For the study of corrosion protection a *Streptomyces* sp. strain was chosen, isolatedon medium 4. To the medium 10% of lactose was added, as it isk nown that at high sugar content some microorganisms, i.e. *Streptomycetes*, can synthesize exopolysaccharides which can protect the metal. The control plate was placed in medium 4 of the tested strain, but without the addition of 10% lactose. Before use the steel panels (10x4x0,2mm) were treated with 70% $C_2H_5OH$ and washed with water. The dimensions of the samples were measured with a micrometer. The results of the experiment were visualized by photographic material.

## 2. RESULTS AND DISCUSSION

### 2.1. Soil Characteristics

The soils were analyzed for pH and moisture content (Table 1). The moisture content of the soils was high at 73,9 – 82,4%, and even higher for plot S6 at 87,7%. pH of the soils was slightly acidic and varied in a relatively narrow range.

**Table1.** *Characteristics of the studied soils*

| Soil sample | *S1* | *S2* | *S3* | *S4* | *S5* | *S6* |
|---|---|---|---|---|---|---|
| **Moisture** | 73,9% | 81,6% | 80,4% | 79,9% | 82,4% | 87,7% |
| **pH** | 6,78 | 6,73 | 6,65 | 6,73 | 6,62 | 6,72 |

After 8 days of culture on the different culture media various microbial colonies were obtained (Table 2). The data showed that the slowest growth among all the samples was recorded on medium M2, regardless of the fact that the medium was rich in organic compounds.Growth on this medium was recorded only for samples S3 and S6. It can be speculated that autotrophic microorganisms dominated the samples which is in line with other microbiological findings for Antarctica.

**Table2.** *Number of obtained colonies*

| Samle/ media | S1 | S2 | S3 | S4 | S5 | S6 |
|---|---|---|---|---|---|---|
| **SSA** | $17.10^5$ | $30.10^5$ | $25.10^5$ | $23.10^5$ | $12.10^5$ | $16.10^5$ |
| **Gauze** | $1.10^2$ | $15.10^3$ | $10.10^3$ | 0 | $9.10^5$ | $20.10^5$ |
| **M5** | $56.10^5$ | $112.10^5$ | $76.10^5$ | $2.10^3$ | $27.10^5$ | $45.10^5$ |
| **M2** | 0 | 0 | $20.10^2$ | 0 | 0 | $23.10^2$ |
| **M4** | $39.10^5$ | $35.10^4$ | $54.10^5$ | $32.10^5$ | 0 | $41.10^5$ |
| **M3** | $123.10^5$ | $98.10^5$ | $40.10^4$ | $78.10^5$ | $20.10^5$ | $16.10^5$ |

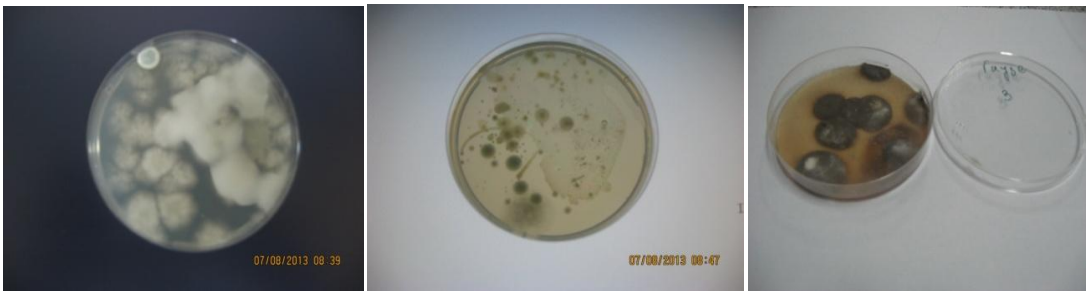
The isolated species of microorganisms were subjected to micro-morphological and macro-morphological studies. The results are presented in Figures 1and 2.

The micro-morphological characteristic suggested that among the isolated species dominated representatives of *Streptomyces sp.* (in samples S1, S2, S3 and S6) and copiotrophic bacteria (in

samples S4 and S5). These results confirmed previous studies on microbial diversity in Antarctica [1,3,6,9].

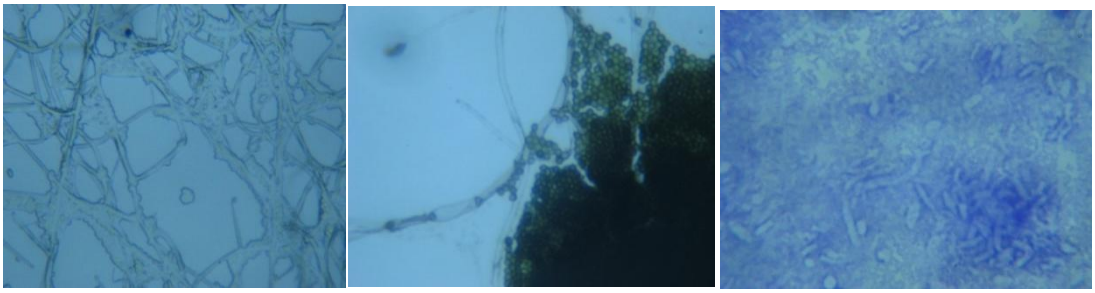
The microscopic pictures also confirmed the presence of high microbial species diversity in some of the samples. It could be clearly seen the presence of diverse structure of the aerial myceliumin two of the isolated species (Fig. 2 A and B), and the cells of the bacteria isolated from sample S5 refer to the Gram positive bacteria.

According to data in the literature among the microorganisms in Antarctica many representatives of the cyanobacteria and the sulphur bacteria are present [12]. Iron- and sulphur reduction are also the main processes in Antarctic marine sediments.



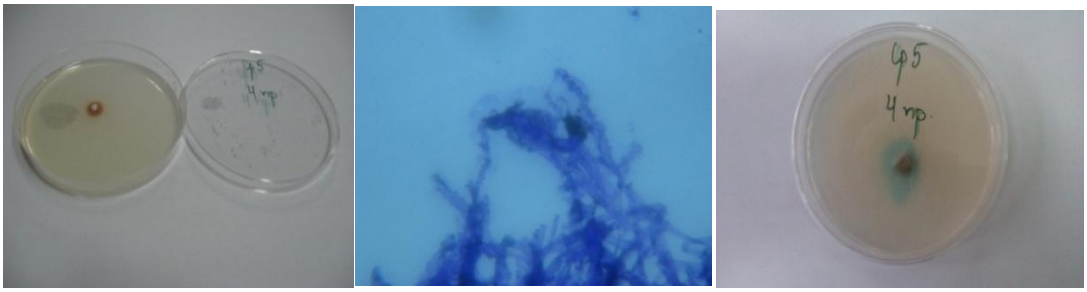
A B C

**Fig1.** *Pictures of the colonies of isolated species: A) Sample S6 on M3; B) SampleS5on SSA; C) SampleS3 on Gause medium*



A B C

**Fig2.** *Light microscopic visualizations of preparation imprint (A, B) and Gram stained (C) of isolated strains: A –sample S6 on M3; B - sample S3 on media Gause; C -sample S5 onSSA. (The pictures were taken using microscope OPTIKA (Italy)at magnification 1000 by immersion).*



A B C

**Fig3.** *Pictures of the bacteria: A – sample S4 on M5; B – light microscopic visualization of the same sample; C – qualitative reaction for Fe (OH)3.*

A colony of a microbial species was isolated from sample S4 on medium M5 which displayed a characteristic rusty color indicating their belonging to the iron bacteria (Fig.3, A). The microscopic picture (Fig.3, B) suggested that the isolated bacteria belonged to *Gallionella sp.*, because of the dichotomically branched filaments characteristic for the species. These filaments are formed as a result of there leased $Fe(OH)_3$ accumulating outside the cell in the bacterial capsule.

Toprove our suggestion we conducted aquality test with a drop of 1% potassium ferrocyanide and a drop of 30% HCl. The characteristic blue color was observed (Fig. 3,C), confirming that the species isolated from sample S4 on M5 medium was amycoplasma belonging to *Gallionella* sp.

One of our aims was to investigate whether some of the isolated microorganisms can be used as corrosion protectors. Arbitrarily we to oka species belonging to *Streptomyces* sp. Isolated on medium M4, but we added a higher concentration of carbohydrate (10%lactose) to the medium. The results obtained are presented in Fig. 4.

A B C

**Fig4.** *Results of the experiment for corrosion protection: A – experimental design; B –sample treated with microorganism; C – control sample.*

The pictures (Fig. 4) clearly showed that the treated with the microorganism sample had no evidence of corrosion (Fig. 4,B) while the control sample, without the addition of lactose, showed strong corrosion (Fig. 4,C). Most probably the tested species of *Streptomyces*sp. Was able to synthesize extracellular exopolysaccharides in media with high concentrations of sugar.

The metallic corrosion represents an economic burden for many industry sectors [12,13]. According to the World Corrosion Organization [14], the annual cost of corrosion is greater than 3% of global GDP (Gross Domestic Product); however, governments and industries pay little attention to corrosion, except in high-risk areas, like aircrafts and pipelines.

In our previous studies [15, 16, 17] it was shown that in the presence of high concentration of lactose (5 to 15 %), high concentration of sucrose 4%, and concentration sucrose 4% and 2% maltose the strains *Lactobacillus delbrueckii B5, L. delbrueckii K27, L. delbrueckii B8, L. delbrueckii O43, L. delbrueckii K3, L. delbrueckii K17, and L. delbrueckii K15* synthesized exopolysaccharides which had corrosion inhibitory properties.

Our study suggested that regard less of the harsh living conditions Antarcticais very rich inspecies of microorganisms belonging to different systematic groups. Many of them can have valuable properties. The isolated species in this study will be further genetically and biochemically investigated.

## ACKNOWLEDGMENTS

This study was funded by project RD-10.03.2015 of Shumen University.

## REFERENCES

[1] Encheva, M., N. Zaharieva, A. Kenarova, N. Chipev, V. Chipeva, P. Hristova, I. Ivanova, P. Moncheva. Abundance and activity of soil actinomycetes from Livingston Island,Antarctica. *Bulgarian Journal of Agricultural Science*, 19 (2) (2013), 68–71.

[2] Carpenter,E, S. Lin, D.Capone. Bacterial activity inSouth Pole snow.*Appl.Environ. Microbiol*. 66,(2000), 4514–4517.

[3] Howard-Williams, C., R. Pridmore, P. Broady, W.Vincent. Environmental and biological variability in theMcMurdo Ice Shelf ecosystem.In: K.R. Kerry, G. Hempel(Eds.), Antarctic Ecosystems: Ecological Change andConservation, Springer-Verlag, Berlin, (1990), pp. 23–31.

[4] Howard-Williams, C, R. Pridmore, M. Downes, W.Vincent. Microbial biomass, photosynthesis and chlorophyllrelated pigments in the ponds of the McMurdo IceShelf, Antarctica. *Antarctic Sci*. 1,(1989), 125–131.

[5] Tang, E.P.Y., R. Tremblay, W. Vincent. Cyanobacteriadominance of polar freshwater ecosystems: are highlatitudemat-formers adapted to low temperature? *J.Phycol*. 33,(1997), 171–181.

[6] Thomas, D.N., G.EFogg, P. Convey, C.H. Fritsen, J.M. Gili, R. Gradinger, J. Laybourn-Parry, K. Reid, D.W.H.Walton(Eds.). The Biology of Polar Regions, Second ed. OxfordUniversity Press, U.K, (2008), 394 pp.

[7] Vincent, W.F., Laybourn-Parry, J. (Eds.). Polar Lakes andRivers e Limnology of Arctic and Antarctic Aquatic Ecosystems.Oxford University Press, 2008, U.K, 327 pp.

[8] Vincent, W.F. Cyanobacteria. In: Likens, G. (Ed.), Encyclopediaof Inland Waters.Elsevier, Oxford U.K.(2009), pp. 226-232.

[9] Vincent,W.F., C. Howard-Williams. Life on snowballEarth.*Science* 287, (2000), 2421.

[10] Vincent, W.F. , D. Mueller, P. Van Hove, C. Howard-Williams. Glacial periods on early Earth and implicationsfor the evolution of life.In: J. Seckbach (Ed.), Origins:Genesis, Evolution and Diversity of Life, Kluwer AcademicPublishers, Dordrecht, The Netherlands, (2004), pp.481–501.

[11] Zakhia, F., A.-D. Jungblut, A. Taton, W. F. Vincent and A. Wilmotte,. Cyanobacteria in cold ecosystems. In: Psychrophiles:from Biodiversity to Biotechnology, Margesin R, SchinnerF, Marx J-C, Gerday C (eds.), Springer-Verlag, BerlinHeidelberg,(2008) pp. 121–135.

[12] Demadis KD, C. Mantzaridis, P. Lykoudis. Effects of structural differences on metallic corrosion inhibition by metalpolyphosphonate thin films. *Industrial & Engineering Chemistry*. 45 (2006) 7795-7800.

[13] Hansson CM. The impact of corrosion on society.*Metallurgical & Materials Transactions*. 42 (2010)2952-2962.

[14] World Corrosion Organization. George FH (Director general). Now is the Time. 2012.

[15] Ignatova-IvanovaTs., Ivanov R, Iliev I. Ivanova I. Study anticorrosion effect of EPS from now strains *Lactobacillus delbruecii*. Biotechnol&Biotechnol EQ Special edition/on line, (2009) 705-708.

[16] Ignatova-Ivanova, Ts., R. Ivanov, I. Iliev, I. Ivanova. Study of the anticorrosion effect of exopolysaccharides produced by *Lactobacillus delbrueckii*B5 cultivated on different carbohydrates. *Biotechnol&Biotechnol EQ* Special edition/on line, (2011) 224-227.

[17] Ignatova-IvanovaTs., R. Ivanov. Anticorrosion effect of biofilm forming *Lactobacillus* strains on metal surfaces. *Bulgarian Journal of Agricultural Science*, 19(2) (2013) 83-85.