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Study Of The Anticorrosion Effect Of Exopolysaccharides Produced By Lactobacillus Fermentum Ts Cultivated On Different Carbohydrates.

Ibryamova S[1], R Ivanov[2], and TS Ignatova-Ivanova[1]*.

[1]Department of Biology, Shumen University, Shumen, Bulgaria Shumen University "Konstantin Preslavski" Department of Biology 115 Universitetska Str., Shumen, Bulgaria.
[2]Department of Organic chemistry and technologies, Shumen University, Shumen, Bulgaria, Shumen University "Konstantin Preslavski" Department of Biology 115 Universitetska Str., Shumen, Bulgaria.

ABSTRACT

Corrosion of metals is a serious and challenging problem faced worldwide by industry. It has been estimated that the yearly corrosion damage costs are currently equivalent to 4.2% of the U.S. gross national product. It was proved that strain Lactobacillus fermentum Ts synthesized exopolysaccharides in the presence of different carbohydrates 10% glucose, 10% sucrose, 10% fructose, 10% galactose, 10% lactose and 10% maltose. The obtained information was used in a study on the anticorrosive properties of exopolysaccharides synthesized by the latter strain. The study of the corrosive stability of steel samples was conducted by using the gravimetrique method. The rate of corrosion, the degree of protection, and coefficient of protection has been calculated. Microscope pictures of the treated steel samples confirmed the corrosive activity. The present research confirms the result that polysaccharides made by microorganisms show anti-corrosive properties. Our data showed that L.fermentum Ts produce EPS, which serve as corrosion inhibitor for mild steel.

Keywords: Corrosion, Inhibitor, Lactic acid bacteria, SEM, AFM

Corresponding author

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INTRODUCTION

Corrosion of metals is a serious and challenging problem faced worldwide by industry. It has been estimated that the yearly corrosion damage costs are currently equivalent to 4.2% of the U.S. gross national product. These costs could be greatly reduced by better and wider use of corrosion protection techniques [1]. Various classes of organic inhibitors, either as herbal or animal extracted form (e.g. starch, chitosan) or bacterial made biopolymer, are successfully used as corrosion inhibitors till date [2]. Literature survey reveals that only limited number of works have been carried out for the corrosion protection of carbon steel in VNSS and saline medium using bacterial made biopolymer [3-5]. Finkenstadt et al. [6] expressed that the anticorrosion properties of bacterial biopolymers were strain-specific. Exopolysaccharides from Lactic acid Bacteria as Corrosion Inhibitors the use of organic coatings to protect metal surfaces through barrier and passivation mechanisms. However, these coatings are not permanent and the cost of applying organic coatings on corroding components in use is extremely prohibitive. Applying coatings before the components are introduced into service involves excessive costs because they are susceptible to abrasions and other forms of mechanically induced damage. Thus, a coating that can be easily applied and maintained on corroding parts and is cost-effective is an attractive alternative to the prevention methods currently in use. Since bacteria can coat metals with a regenerative biofilm, it is becoming evident that they may be used as a means of preventing corrosion [7-10].

In this paper, data on the effect on corrosion of steel of EPS produced by Lactobacillus fermentum Ts, cultivated on media with different carbohydrates are presented and discussed.

MATERIALS AND METHODS

Strain: Strain Lactobacillus fermentum Ts was obtained from the collection of the Department of Biology, Shumen University. Molecular analysis in LAB (lactic acid bacteria) was performed by molecular identification (16S rRNA gene sequencing) in GeXP Genetic Analysis System (Beckman Coulter, USA) [11].

Media: The strain cultivated in media of MRS (de Mann Rogosa Sharpe, Biolife 272-20128, Milano, Italia) in composition, g/L: Tween 80—1; pepton from casein—10.0; meat extract—8.0; yeast extract—4.0; K_2HPO_4—2.0; sodium acetat—5.0; amonium citrate—2.0; $MgSO_4 \cdot 7H_2O$—0.2 and $MnSO_4$—0.05. The pH of media was adjusted to 6.5 with 1 M NaOH. The basic media was sterilized by autoclaving at 121 °C for 20 min, and carbohydrates supplemented were sterilized using 0.22 μM filters (Manisart®). The basic MRS broth was supplemented with 10% glucose, 10% sucrose; 10% fructose, 10% lactose, 10% galactose and 10% maltose to be tested.

Media for study of microbial biofilm with congo red agar CRA: The strain cultivated in media of MRS (de Mann Rogosa Sharpe, Biolife 272-20128, Milano, Italia) with 10% glucose,10% sucrose, 10% fructose, 10% galactose, 10% lactose and 10% maltose and congo red. CRA plates were inoculated with test organisms and incubated at 37° C for 24 h aerobically. Black colonies with a dry crystalline consistency indicated biofilm production [12]. The experiment was performed in triplicate and repeated three times.

STUDY OF THE CORROSIVE STABILITY

The study of the corrosive stability of steel samples was conducted with the gravimetrique method [13]. Before use, steel panels (10 × 4 × 0.2 mM) were treated with 70% C_2H_5OH, washed with water and dried in an oven, cooled in a desiccators, weighed on a balance and kept in a desiccators unit used. The weight of the samples was measured using analytical balances. The dimensions of the samples were measured with micrometer. Six types of experimental series were performed:

(a) cultivation of the studied strain in mMRS media with 10 % of glucose;
(b) in mMRS media with 10% fructose;
(c) in mMRS media with 10% sucrose;
(d) in mMRS media with 10% galactose;
(e) in mMRS media with 10% lactose;
(f) in mMRS media with 10% maltose.

Initially the steel samples were added in two variants: deproteinised supernatant and free cell supernatant. Then the steel samples were added in HCl as control probe and a dilution (3: 100) of the cultural media of the studied strain was added as inhibitor of the corrosion. The duration of the procedure was 120 h at 18 °C. After the treatment the steel samples were washed with water and dried to constant weight. The structure of layer over steel plates was analyzed by SEM (scanning electron microscopy) JSM 5510.

PARAMETERS OF CORROSION

After retrieval, the corrosion products were removed when washed with water. They were dried in an oven. After the removal of corrosion, steel plates were cleaned and reweighed as above to estimate weight loss. The rate of corrosion, the degree of protection, and coefficient of protection were calculated.

The corrosion rate K (g/cm2·h) was presented as follows: $K = \Delta G / S \cdot \tau$ (1)

Where, Δ is the corrosion rate; ΔG—losses of mass consequence of corrosion, g; S—is the area of plates, m2; τ—is duration of the corrosion, h.

In order to track out the inhibitor properties of EPS synthesized in media, the degree of protection (Z) and coefficient of protection (γ) have been calculated using the formulas:

$$Z = (K0 - Ki) / K0 \times 100, \% \quad (2)$$

$$\gamma = K0 / Ki \quad (3)$$

Where, K0 is the corrosion rate in control media; Ki—the corrosion rate in test media

ANALYSIS BY MEANS OF SEM MICROSCOPY

The steel plates made of low carbon steel are weighe\d with an allowance of 0,0001g with an assay-balance. They are put sterilely in a liquid ambient which contains a L. fermentum Ts. The samples were incubated at 37°C for 24 h. The structure of the layer over the metal plates was analyzed by SEM (scanning electron microscopy) JSM 5510 and atomic force microscopy (AFM) . All experiments were performed in triplicate [14].

ANALYSIS BY MEANS OF AFM MICROSCOPY

An AFM Anfatec Instruments AG, Germany was used for characterization of surface topology. The measurements were realized in non-contact mode when the tip was scanning over the studied surface at a distance of few nanometers. A silicon nitride tip with a curvature radius of about 10 nm and force constant about 43 N/m was used. The three-dimensional images of scanned samples were created with ANFATEC PRESENT software.

RESULTS AND DISCUSSION

The presence of EPS associated with bacterial cells can be recognized by the formation of colonies in mucous solid medium [15]. Therefore, the presence of a translucent or creamy material involving a mucoid colony is indicative of EPS production potential. When cultivated in a media with high content of saccharides such as 10% sucrose solutions, 10% fructose solutions, 10% glucose solutions, 10% galactose solutions, 10% lactose solutions and 10% maltose solutions, strain L. fermentum Ts synthesizes exopolysaccharides (Fig. 1).

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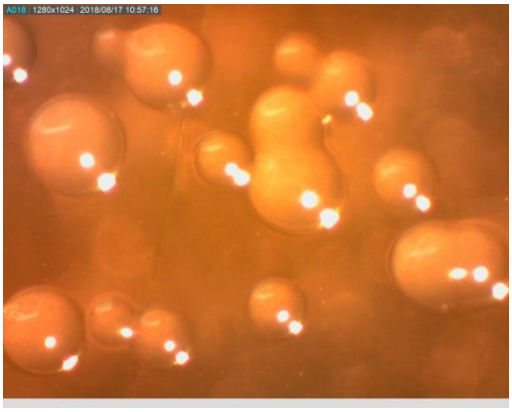

Fig 1: EPSs (exopolysaccharides) produced by L. fermentum Ts cultivated in a media containing 10% maltose, which are secreted in the culture medium.

The pictures were taken using stereomicroscope OPTIKA (Italy).

For chemical analysis of the formed microbial biofilm was used congo red agar CRA method and the results are represented on Figure 2.

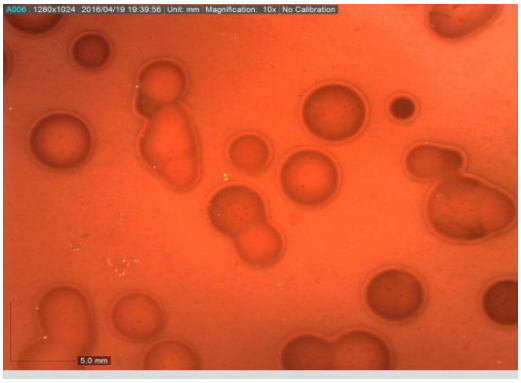

Figure 2: EPSs (exopolysaccharides) produced by L. fermentum Ts Congo red agar Black colonies shows biofilm formation of LAB, cultivated in media contained 10% maltose. The pictures were taken using stereomicroscope OPTIKA (Italy).

In our previous studies [16-21], it was shown that at the presence of high concentration of lactose (5% to 15%), high concentration of sucrose 4%, mixed sucrose 4% and 2% maltose and mixed sucrose 5% and 5% maltose, mixed 5% sucrose and 5% fructose and mixed 5% sucrose and 5% fructose 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 have inhibitory properties.

Strain L. fermentum Ts was cultivated in a media containing 10% sucrose,10% glucose, 10% lactose, 10% fructose, 10% galactose and 10% maltose for 12 h. The steel samples were placed in seawater as control probe and a dilution (3: 100) of the cultural media of the studied strain was added as inhibitor of the corrosion. The received results are presented in Table 1 and figure 3.

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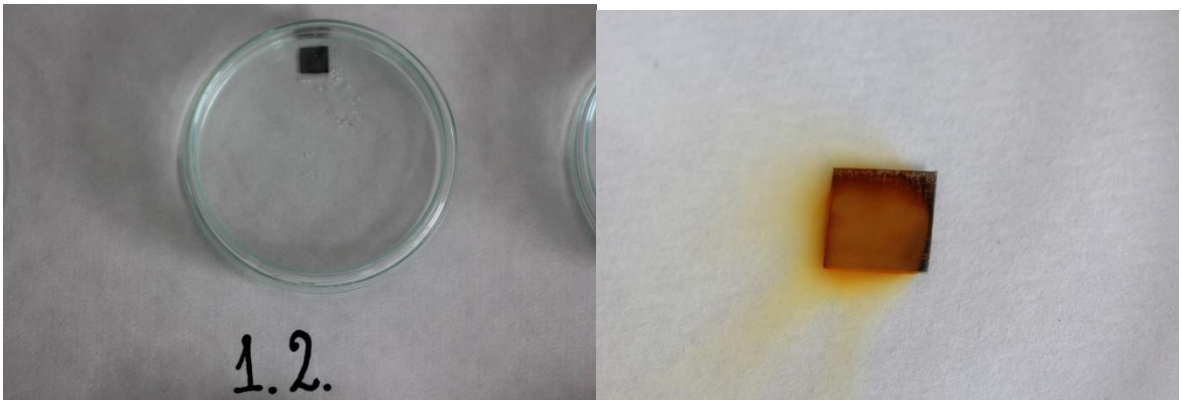

A) B)

Fig 3: Steel samples immersed in HCl for a period of 120 h.
(A) Steel plates in HCl with inhibitor supernatant obtained of mixed 10% maltose; (B) control—steel plates in HCl.

Table 1: Characterization of the protective properties in HCl with added supernatant.

№ Sample	media	The quantity of the supernatant in Seawater, %	K.10^{-4}, g/cm^2.h	Z, %	γ
1.	10% glucose	3.0	2.242	**43.07**	1.76
2.	10% fructose	3.0	2.560	34.97	1.54
3.	10% sucrose	3.0	2.535	35.61	1.55
4.	10% galactose	3.0	2.558	35.03	1.54
5.	10% lactose	3.0	2.775	29.52	1.42
6.	10% maltose *	3.0	2.445	37.88	1.61
7.	control	-	3.937	-	-

*The steel plates were photographed after washing; Results are mean ± SEM of three separate trails.

From the presented data in Table 1 the protective effect in all studied cases is visible. The coefficient of the protection of corrosion varied between 1.76 and 1.42. The efficiency of the inhibition of corrosion was higher in the presence of 10% glucose were used.

When used as inhibitor of the protection strain L. fermentum Ts cultivated in the presence of 10% glucose the protection of corrosion was highest (table 1). It could be underlined that 10% carbohydrates in the media stimulated the process of protection of corrosion.

The structure of the layer over the steel plates was analyzed by Scanning electron microscopy. The results from this procedure are shown in Fig. 4.

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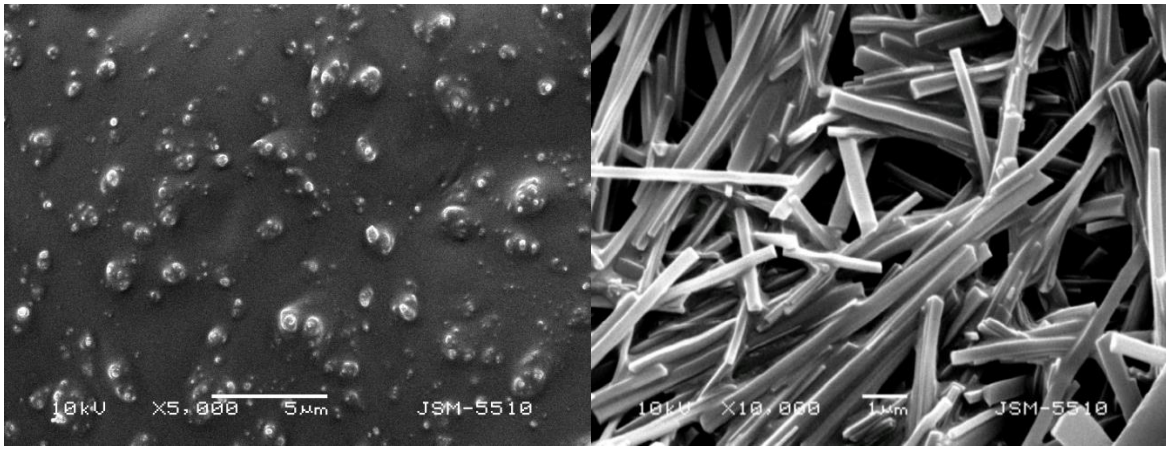

A) B)

Fig 4: Biofilm formed by L. fermentum Ts on the surface of mild steel, visualized using SEM. (A) Steel plates after corrosion in seawater with inhibitor supernatant obtained of mixed 10% maltose; (B) control—steel plates after corrosion in HCl.

The biofilm makes it not easily corrodible in seawater, supplemented with cultivated ambient from the same strain grown in a composite of 10% glucose (figure 4A). Figure 4B shows a picture of a steel surface sample treated directly with seawater. The observed lamellae are most probably $FeCl_2$ crystals, product of the corrosion. Microscope techniques provide information about the morphology of microbial cells and colonies, their distribution on the surface, the presence of EPS (Fig. 4A) and the nature of corrosion products (crystalline or amorphous; Fig. 4B). They can also reveal the type of attack (e.g. pitting or uniform corrosion) by visualizing changes in microstructure and surface features after removal of the biofilm and corrosion products (Fig. 4B).

AFM imaging of biopolymer as polysaccharides was generally conducted in air or under a liquid in order to avoid excessive dehydration. The topographical AFM images of L. fermentum Ts EPS were shown in Fig.5.

The SEM images of the EPS showed a stacked flakes with relatively uniform shapes Fig.4A. Similar porous web microstructure of EPS was reported earlier with the EPSs produced by Streptococcus thermophilus GST-6 [25] and Lactobacillus plantarum strains [26-27]. Chen M., and colleagues [28] showed that after sulphonation the appearance of polysaccharide fragments without uniform size could be changed into regular even structure similar to tile. This was in agreement with the similar micro structural change described above for the EPS from strain L. fermentum Ts of this study. However, Zhang and colleagues [29] demon strated a polyphasic convoluted structure by SEM of a sulphated persimmon polysaccharide. Sulphonation of polysaccharides, involving both dehydrolysis and hydrolytic degradation [30], could change appearance of the polymers. These reactions resulting in solubility change of the polysaccharides [31], together with the etch effect of hydrolysis during sulphonation were thought to be important factors affecting appearance of sulphated polysaccharides. However, the molecular mechanism of how sulphonation affecting micro structure of EPS needs to be further studied.

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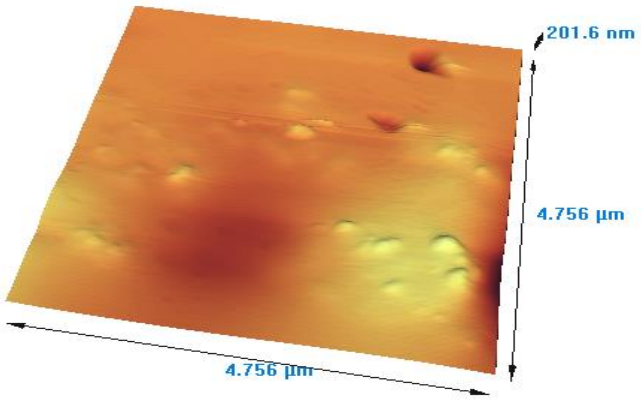

Figure 5: Atomic force microscopy (AFM) images of molecular structure of L. fermentum Ts EPS. Biofilm formed by lactic acid bacteria cultivated with 10% maltose.

L. fermentum Ts EPS deposited from 10 g/mL aqueous solution, roundness lumps and chains can be seen (Fig. 5).

The AFM-based single-molecule force spectroscopy (AFM–SMFS) technology is a powerful tool to characterize the force-induced conformational transitions, the dynamics, and super molecular structures of polysaccharides at the molecular level [32-34]. The maximal height of lumps at 10% solution of glucose is 166,2 nm, at 10% solution fructose is 193nm and 10% solution maltose is 201,6nm. This result suggested that L. fermentum Ts EPS could combine water in the aqueous. Furthermore, it showed pseudo plastic behavior because the strong interaction between water molecules and the hydroxyl groups (−OH) of L. fermentum Ts EPS. A similar experiment was reported about an acidic polysaccharide from Mesona blumes gum [35-36]. The AFM images of Mesona blumes gum showed different shapes, spherical lumps and worm, respectively in low and high concentration. The reduction in viscosity could also be attributed to polymer degradation due to the cleavage of glyosidic bonds with in the polysaccharide structure [37].

The forms of corrosion which can be promoted by the interaction of microorganisms with metals are numerous, including general pitting, crevice attack, stress corrosion cracking, enhancement of corrosion fatigue, intergranular stress cracking and hydrogen embrittlement and cracking. Most cases of microbially-influenced corrosion (MIC) are associated with localized attack. The complexity of MIC reactions means that a broad range of techniques must be employed to relate the corrosion processes to the microbial activities at surfaces [38]. The role of EPS in MIC of stainless steel remains obscure. It has been postulated that they are not sufficient to induce biocorrosion of stainless steel unless aided by the presence of a biocatalyst of oxygen reduction. Lai, M.E [39], showed that which could be oxido-reductase enzymes entrapped in the biofilm. EPS has even been suggested to protect metal surfaces from corrosion.

CONCLUSION

From the received results it was evident that a mixture of 10% sucrose,10% glucose, 10% lactose, 10% fructose, 10% galactose or 10% maltose maltose stimulated the formation of microbial biofilm inhibiting the corrosion of steel. The present research confirms the result that polysaccharides made by microorganisms show anti-corrosive properties. Especially, homopolysaccharides showed interesting results for the protection of steel. Measurements indicate that it takes some time for layers of biopolymers on the metal to build a complete protective layer. Our data showed that L. fermentum Ts produce EPS, which serve as corrosion inhibitor for mild steel. Further studies are needed to evaluate the potential of the biofilm exopolysaccharides as anticorrosive agents.

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