

Corrosion protection of low-carbon steel using exopolysaccharide coatings from *Leuconostoc mesenteroides*

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Abstract Corrosion of metals is a serious and challenging problem faced worldwide by industry. Purified *Leuconostoc mesenteroides* exopolysaccharide (EPS) coatings, cast from aqueous solution, inhibited the corrosion of low-carbon steel as determined by electrochemical impedance spectroscopy (EIS). There were two different corrosion behaviors exhibited when EPS films from different strains were cast onto the steel. One EPS coating reacted immediately with the steel substrate to form an iron (III) oxide layer (“rust”) during the drying process while another did not. The samples that did not flash corrode had higher corrosion inhibition and formed an iron (II) passivation layer during EIS testing that

persisted after the cells were disassembled. Corrosion inhibition was strain-specific as polysaccharides with similar structure did not have the same corrosion potential.

Keywords Corrosion · Exopolysaccharide · *Leuconostoc mesenteroides*

Introduction

Corrosion of metals is one of the most serious and challenging problems faced by industries worldwide. When metals come in contact with different environments, such as air, water, chemical products or pollutants, they begin to degrade from interaction of the metal with its environment. Corrosion causes severe damage to industrial equipment, materials and buildings, and as a result, incurs additional costs such as painting, coatings, premature replacement of materials due to degradation, increased maintenance and more expensive, corrosion-resistant materials. The adoption of preventive measures that reduce or eliminate corrosion is financially costly and time consuming. In fact, corrosion prevention and treatment consume more than 20% of a typical industrial budget. The National Institute of Standards and Technology (NIST) estimated in 1996 that the cost of corrosion for the United States is over \$300 billion (Bennett et al. 1996). Current corrosion protection

Disclaimer: Names are necessary to report factually on available data. However, the United States Department of Agriculture neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval to the exclusion of others that may also be suitable.

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measures usually involve toxic or expensive applications such as volatile organic compounds (VOCs) that are considered to be a major human health issue.

Accelerated corrosion in which metal substrates were colonized by bacteria had been noted (Beech and Sunner 2004; Beech and Gaylarde 1991). However, in some cases, biofilms, composed of a secreted polymeric substance containing microbial populations, have shown to inhibit corrosion in metals (Jayaraman et al. 1997; Chongdar et al. 2005; Fang et al. 2002; Majumdar et al. 1999). In most cases, the metal was submerged in an aqueous medium inoculated by a bacterial species. The bacteria colonized the metal substrate and produced a heterogeneous extracellular polymeric substance that adhered to metal surfaces facilitated by the functional groups of the exopolymer substance. Corrosion inhibition was usually measured by loss-in-weight calculations and generally attributed to the diffusional barrier provided by the bacterial biofilm.

Biofilms are primarily polysaccharides but also may contain proteins, fatty acids and other small molecules. Work by Stadler et al. (2008) concentrated on corrosion by sulphate-reducing bacteria but suggested that dextrans could prevent corrosion on metals. Van Leeuwen et al. (2008) found an $\alpha(1 \rightarrow 3)$, $\alpha(1 \rightarrow 6)$ -linked D-glucan produced by *Lactobacillus reuteri* that inhibited corrosion while dispersed in a electrolyte solution rather than as a coating (Penninga et al. 2002; van Leeuwen et al. 2008).

While corrosion resistance of some biopolymers has been suggested by anecdotal data and poorly refined electrochemical data, there has not been an electrochemical analysis of corrosion behavior in metals coated with EPS from *Leuconostoc mesenteroides*.

Materials and methods

Materials and cultures

Purified exopolysaccharides produced from bacteria that have commercial applications were dispersed in nanopure water. Specimens are listed in Table 1. *Leuconostoc mesenteroides* NRRL strains B-1355 and B-1498 were grown as previously described using cell-free culture fluids to produce glucans from sucrose (Cote and Robyt 1982). The glucans were

precipitated from reaction mixtures with ethanol (Wilham et al. 1955). These strains in their native state synthesize two glucans, referred to as fraction L (Less soluble, precipitated at 38% v/v ethanol) and fraction S (Soluble, precipitated at 46% v/v ethanol). L means less soluble in ethanol/water mixtures, S means more soluble in the mixtures. This is the convention introduced by Jeanes et al. (1954) and is in common usage. Fraction L is predominantly $\alpha(1 \rightarrow 6)$ -linked D-glucose, with approximately 3–4% branching through $\alpha(1 \rightarrow 3)$ -linkages. Fraction S, better known as alternan, consists of an alternating sequence of $\alpha(1 \rightarrow 3)$ and $\alpha(1 \rightarrow 6)$ -linked D-glucopyranosyl units with approximately 11% branching (Leathers and Cote 2008). Both are high-molecular weight ($>10^6$ g/mol), water-soluble D-glucans. The fraction S samples (alternan) from strains B-1355 and B-1498 differ in their proportions of each linkage type, with B-1498 Fraction S containing a relatively greater proportion of $\alpha(1 \rightarrow 6)$ -linkages (Seymour et al. 1979). Phosphomannan was prepared and characterized previously (Seymour et al. 1976). These samples were lyophilized. Levan is a naturally occurring polymer of β -D-fructofuranose with $\beta(2 \rightarrow 6)$ linkages between fructose rings and branching at C-1 (Barone and Medynets, 2007). Levan (mol wt $>10^6$ g/mol) produced from *Bacillus* sp. was obtained from Montana Polysaccharides. The levan was in powder form.

Polysaccharide samples (0.5 g) were dissolved in approximately 20 ml nanopure water. Samples were cast onto SAE 1010 low carbon, cold rolled steel panels (Type R35, Q-Panel Lab Products) and allowed to dry overnight at room temperature (20°C) and humidity (30% RH). Thickness was measured by using a MiniTest 2100 (ElektroPhysik). Samples were typically about 100 μ m thick.

Analyses

Polymeric films were evaluated for initial flash corrosion on metal substrates and corrosion inhibition. Electrochemical measurements were performed using a PARSTAT 2273 Advanced Potentiostat (Princeton Applied Research). Electrochemical impedance was recorded at an AC voltage of 10 mV for a frequency range of 100 kHz and 10 mHz. The test cell was a Gamry PTC1 Paint Test Cell (Gamry Instruments) equipped with a conventional three electrode system

Table 1 Structure information for potentially anti-corrosive polysaccharide coatings

Exopolysaccharide	Source description	Monomers	Linkages backbone, branch ^a	Reference
B1498L	<i>Leuconostoc mesenteroides</i> NRRL B-1498, fraction L, dextran	α -D-glucose	$\alpha(1 \rightarrow 6)$, $\alpha(1 \rightarrow 3)$	Seymour et al. (1979)
B1498S	<i>Leuconostoc mesenteroides</i> NRRL B-1498, fraction S, alternan	α -D-glucose	$\alpha(1 \rightarrow 3)(1 \rightarrow 6)$ (alternating)	Seymour et al. (1979)
B1498 N	<i>Leuconostoc mesenteroides</i> NRRL B-1498, mixed native fraction	α -D-glucose		Jeanes et al. (1948, 1954)
B1355L	<i>Leuconostoc mesenteroides</i> NRRL B-1355, fraction L, dextran	α -D-glucose	$\alpha(1 \rightarrow 6)$, $\alpha(1 \rightarrow 3)$	Seymour et al. (1976)
B1355S	<i>Leuconostoc mesenteroides</i> NRRL B-1355, fraction S, alternan	α -D-glucose	$\alpha(1 \rightarrow 3)(1 \rightarrow 6)$ (alternating)	Cote (2002)
B1355N	<i>Leuconostoc mesenteroides</i> NRRL B-1355, mixed native fraction	α -D-glucose		Jeanes et al. (1948, 1954)
B512F	<i>Leuconostoc mesenteroides</i> NRRL B-512F, commercial dextran	α -D-glucose	$\alpha(1 \rightarrow 6)$, $\alpha(1 \rightarrow 3)$	Leathers (2002a)
Y2448	<i>Pichia (Hansenula) holstii</i> NRRL Y-2448, phosphomannan	α -D-mannose Phosphate	$\alpha(1 \rightarrow 3)$, $\alpha(1 \rightarrow 2)$	Seymour et al. (1976)
Levan	Proprietary <i>Bacillus</i> sp.	β -D-fructose	$\beta(2 \rightarrow 6)$, $\beta(2 \rightarrow 1)$	Rhee et al. (2002)
Pullulan	<i>Aureobasidium pullulans</i>	α -D-glucose	$\alpha(1 \rightarrow 4)$, $(1 \rightarrow 4)$, $(1 \rightarrow 6)$ (trimer)	Leathers (2002b)
B1459	<i>Xanthomonas campestris</i> NRRL B-1459, xanthan	β -D-glucose α -D-mannose β -D-mannose β -D-glucuronic Acetate Carboxyethylidene	$\beta(1 \rightarrow 4)$	Jansson et al. (1975)

^a Branch point is defined as di-substituted sugar

with a saturated calomel reference electrode and a graphite counter-electrode. The test area was approx. 14 cm² and NaCl (5% w/v) and H₂SO₄ (0.5 M) were used as electrolyte solutions. Analysis was performed using an electrochemistry software including modules specifically designed for AC and DC corrosion measurements and analysis of Tafel, Nyquist, and Bode plots (Princeton Applied Research). Pore resistance is a measurement of the penetration of an electrolyte through the film and its subsequent

reaction at the metal surface. Pore resistance, as measured from the low frequency domains of electrochemical impedance, was calculated and the relative effectiveness of the coatings was determined.

Results and discussion

Casting of aqueous coatings brought the metal into direct contact with water, or more properly, the

polymer solution. Film formation depends upon water evaporation, polymer coalescence and adherence to the metal substrate. Flash corrosion (or rust)

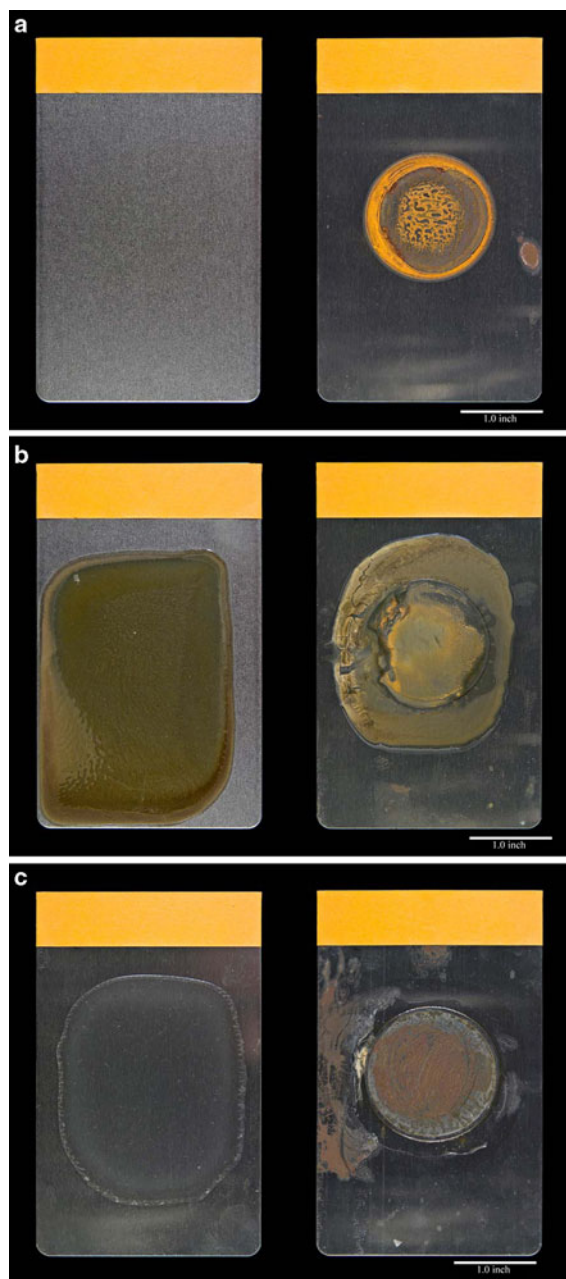


Fig. 1 **a** Control low carbon steel panel before (*left*) and after (*right*) electrochemical evaluation with 5% (w/v) NaCl corrosive agent. **b** Low carbon steel panel coated with EPS-B1355 before (*left*) (*right*) electrochemical evaluation with 5% (w/v) NaCl corrosive agent. **c** Low carbon steel panel coated with EPS-B1498L before (*left*) and after (*right*) electrochemical evaluation with 5% (w/v) NaCl corrosive agent

is considered a defect on metal substrates that appears almost exclusively on the application of water sensitive coatings (Kalendova 2002). Flash corrosion is observed in high humidity environments even with coatings cast out of organic solvents therefore a water-dispersible coating would be expected to flash corrode. However, EPS-B1498L dried without any flash corrosion, while the other EPS samples had a reddish brown pigment under the coating and on the metal surface. After 60 days in normal lab environment, the metal substrate coated with EPS-B1498L still had no corrosion evident, and the film maintained its integrity. Figure 1a shows a control sample of SAE 1010 steel before and after testing under 5% (w/v) NaCl electrolyte solution. Figure 1b represents

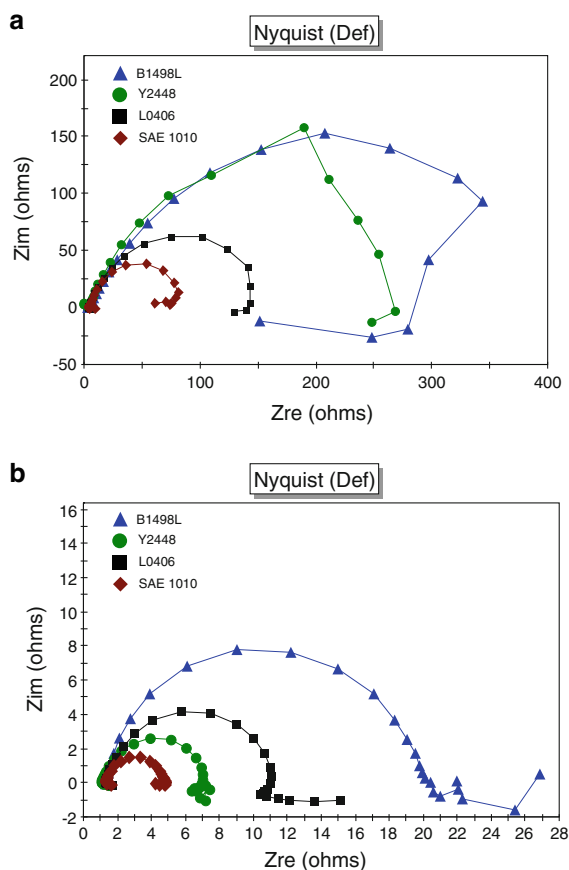


Fig. 2 **a** Complex impedance for selected exopolysaccharide coatings with 5% (w/v) NaCl as the electrolytic solution. The diameter of Zre (x-axis) corresponds to the relative anticorrosive inhibition of the sample. **b** Complex impedance for selected exopolysaccharide coatings with 0.5 M H₂SO₄ as the electrolytic solution. The diameter of Zre (x-axis) corresponds to the relative anticorrosive inhibition of the sample

the typical reaction of SAE 1010 steel after coating with EPS systems, in this case, B1355. The formation of flash corrosion occurred within a few minutes after casting. The corrosion under the coating was heterogeneous and appeared reticulated. The EPS-B1498L, however, dried with no flash corrosion (Fig. 1c) and produced the best quality film of all the samples. The ability of EPS-B1498L to prevent corrosion was puzzling because similarly structured dextrans produced by a different strain, B1355, did not yield similar results. Samples of similar exopolysaccharides (B1498S, B1498N, B1355L, B1355S, B1355N, see Table 1) were collected and tested but not to be as good as EPS-B1498L. Other samples of bacterial EPSs, phosphomannan (Y2448), a fructopolysaccharide (L0406 “levan”), xanthan (B1459)

and commercially available dextrans (B512F), were obtained and evaluated—see Table 1. EPS which formed good coatings (smooth to the touch, non-tacky, and no bubbles or cracks) were evaluated using EIS. The thickness of the coating was approximately 100–130 μm . EPS-B1498L did not flash-corrode and provided the best protection from the corrosive environment of all EPS evaluated in this study. The EPS coatings selected for comparison were EPS-Y2448 and EPS-L0406. These two EPSs produced the least amount of flash corrosion and had suitable if not ideal coating integrity. EPS-1498S and EPS-1355N had similar complex impedance spectra to EPS-Y2448 and EPS-L0406, respectively, but exhibited more variability at low frequency due to breakdown of the films and subsequent corrosion of

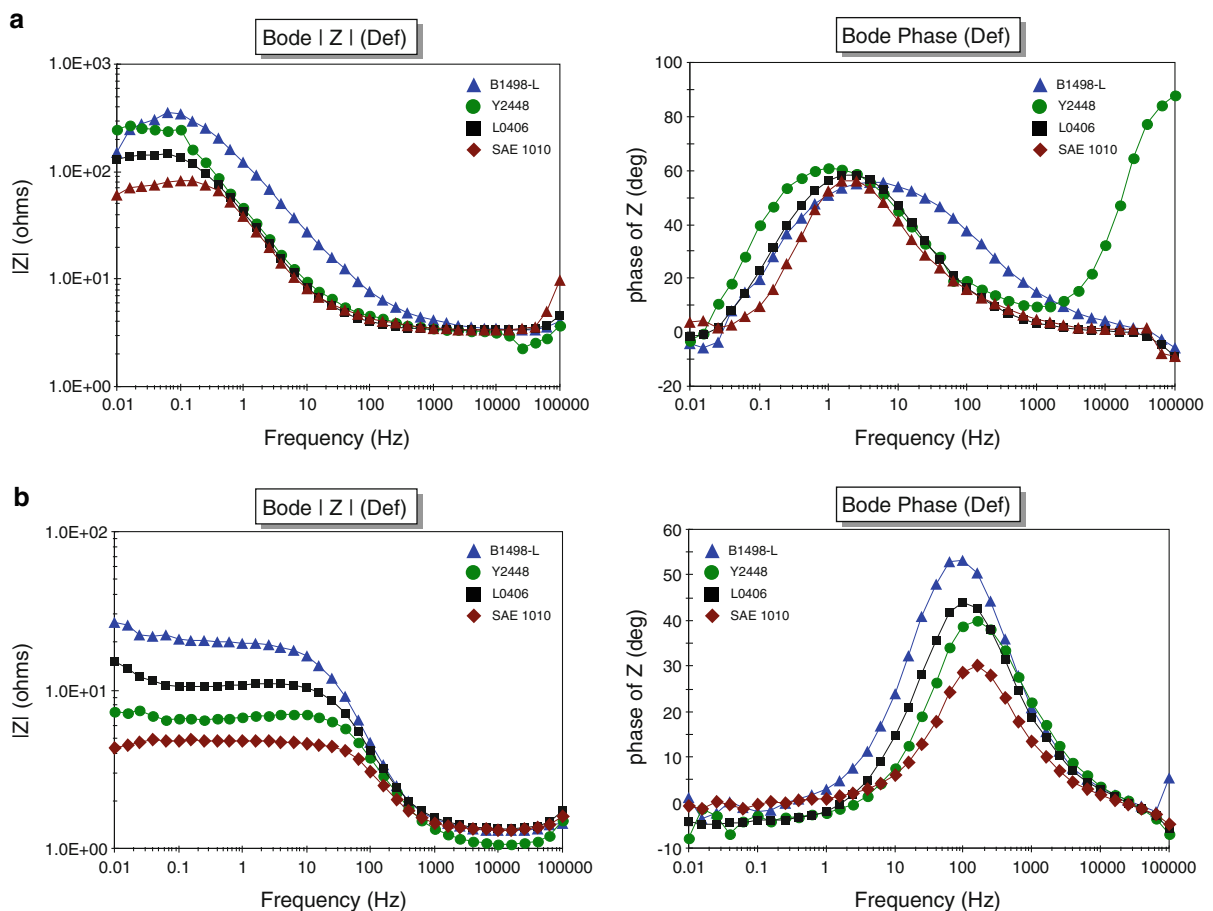


Fig. 3 **a** Bode plots for selected exopolysaccharide coatings with 5% (w/v) NaCl as the electrolytic solution. **a** Magnitude of Z and **(b)** phase of Z . The shift towards higher frequency indicates higher corrosion inhibition by the sample. Complex impedance plots are constructed from Bode plots. **b** Bode plots

for selected exopolysaccharide coatings with 0.5 M H_2SO_4 as the electrolytic solution. **a** Magnitude of Z and **(b)** phase of Z . The shift towards higher frequency indicates higher corrosion inhibition by the sample. Complex impedance plots are constructed from Bode plots

the metal substrate. The order of protection was: B1498L > > Y2448 (1498S) > > L0406 (1335N) > > steel.

Figure 2a shows the complex impedance of bacterial exopolysaccharides on SAE 1010 steel with NaCl as the electrolyte solution. The larger the diameter of the loop, Zre, the better the corrosion inhibition of the coating. The Bode plots for magnitude and phase angle are shown in Fig. 3a. The phase angle versus frequency clearly showed that EPS-B1498L is shifted towards the higher frequencies when compared to SAE1010 steel and the other EPSs. Samples that were tested under a corrosive environment had a disturbed coating and exhibited accelerated rusting when exposed to normal atmosphere in the lab except for EPS-B1498L which was coated with a fine layer of black pigment which slowly gave way to rust after about 3 days. Under a more corrosive environment (0.5 M H₂SO₄), EPS-B1498L provided the best protection and L0406 was transposed with Y2448 for corrosion protection (B1498L > > L0406 > > Y2448 > > steel). The Nyquist plot and Bode plots are shown in Figs. 2b and 3b, respectively. Under a more corrosive environment, the change in magnitude and phase angle is more pronounced (Fig. 3b). The change in the real axis (x) between NaCl (Fig. 2a) and H₂SO₄ (Fig. 3a) which indicates the corrosive nature of the electrolyte solution, should be noted.

The adherence of EPS films to metal surfaces is facilitated at least in part by the interaction of metal ions and the functional groups of the polymers. This interaction could significantly alter the redox potential of Fe(II)/Fe(III). Corrosion of steel begins with the anodic oxidation of Fe to Fe(II). Fe(II) undergoes further oxidation to Fe(III) which then accelerates the conversion of Fe to Fe(II). The metal-EPS system, therefore, can inhibit corrosion by reducing the amount of electron acceptors at the interface by binding Fe(II) and Fe(III). Visual observation of the corroding system indicated the formation of Fe(II) oxide (black) in the EPS-B1498L system while the flash corrosion present in the other EPS samples contained Fe(III) oxide (orange), both which were bound into the EPS and could be removed by peeling back the EPS layer. The formation of Fe(II) oxide was noted during a simple immersion test in a

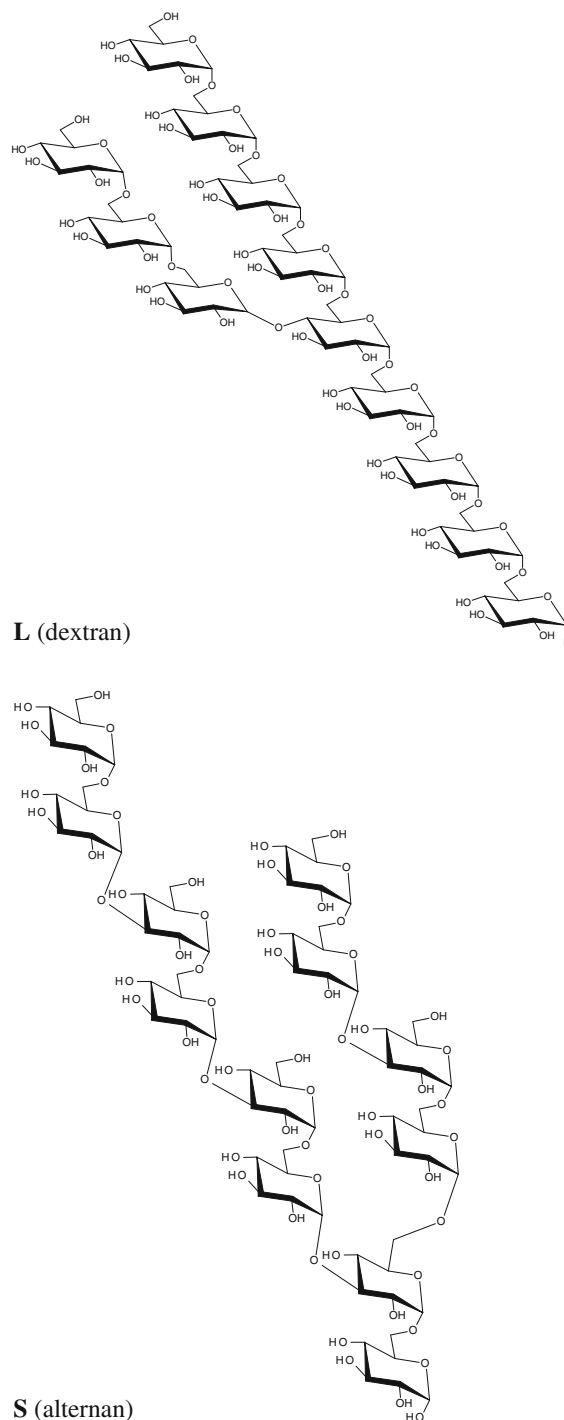


Fig. 4 Statistical structures of S and L fractions of dextran produced by B1498 and B1355 strains of *Leuconostoc mesenteroides*. They have similar structure but different anticorrosion properties

solution of dextran produced by a lactobacillus which was found to have similar structure to EPS-B1498S (Penninga et al. 2002; van Leeuwen et al. 2008). We explored the possibility that residual reducing sugars, specifically fructose, could be responsible for the corrosion inhibition by spiking dextran samples with small amounts (<1%) and then casting onto SAE1010 steel substrates. The addition of fructose stopped the film-forming capabilities of the EPSs and produced flash corrosion. Figure 4 shows a statistical structure of dextrans produced from B1498 and B1355 bacteria strains as well as the reported structure for EPS180 (van Leeuwen et al. 2008). The L fraction consists of $\alpha(1 \rightarrow 6)$ linked D-glucose units with $\alpha(1 \rightarrow 3)$ branching occurring in approximately 1 out of 25 glucose units. The S fractions (alternan) are more highly branched (1 in 10) and have alternating $\alpha(1 \rightarrow 3)$ and $\alpha(1 \rightarrow 6)$ linkages along the backbone. If vicinal diols are influencing the Fe redox potential, the L fractions have significantly more diols available (2,3 and 3,4 diols) than S fractions; however, EPS-B1355L did not exhibit similar behavior as EPS-1498L; therefore the anti-corrosion properties of exopolysaccharides appeared strain-specific.

Conclusions

Corrosion inhibition appears to be strain-specific with dextran-producing bacteria such as *Leuconostoc mesenteroides*. In the case of exopolysaccharides, the structure of the film as it is deposited on the substrate may play a role in its corrosion potential. Similarly, the adhesion of the coating to the substrate or the diffusion of corrosive species through the coating may also play a role. Investigations are underway to determine film formation, adsorption phenomenon, and diffusion parameters for EPS coatings on metal substrates.

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