Article:
Wang, S, Redmile-Gordon, M, Mortimer, M et al. (5 more authors) (2019) Extraction of extracellular polymeric substances (EPS) from red soils (Ultisols). Soil Biology and Biochemistry, 135. pp. 283-285. ISSN 0038-0717

https://doi.org/10.1016/j.soilbio.2019.05.014

Extraction of extracellular polymeric substances (EPS) from Red Soils (Ultisols)

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The authors declared there is no conflict of interest.

Abstract

Extracellular polymeric substances (EPS) have many beneficial functions in soils. Accurate quantification of EPS in soils is crucial. Here, five methods were compared for their suitability for extraction of EPS from Ultisols: hot water extractable polysaccharide (HWEP), hot dilute acid extractable polysaccharide (HDAEP), easily extractable glomalin (EEG), sodium sulfide (SS) and cation exchange resin (CER) method. Humic-acid equivalent (HAE) was used as an indicator for extracellular contamination and ATP for quantifying intracellular contamination from cell lysis. Among the tested methods, CER resulted in EPS extraction with minimal contamination. Therefore, we propose that CER is currently the most appropriate method for extraction of EPS from Ultisols.

Key words: Soil biofilms; Extracellular polymeric substances; Polysaccharide; Cation exchange resin; Easily extractable glomalin

In soils, many microbes are found existing in colonies or biofilms (Deng et al., 2015). The cells in biofilms are embedded in a matrix of extracellular polymeric substances (EPS). EPS is primarily composed of polysaccharides and proteins, but also contains DNA and other constituents (Sheng et al., 2010). Although EPS represents a relatively minor component of soils, it has been shown to have beneficial functions in soils. EPS can protect microorganisms against biotic and abiotic stress (Or et al., 2007), improve water retention (Adessi et al., 2018), and enhance formation and stability of soil aggregates (Bezzate et al., 2000; Büks and Kaupenjohann, 2016;

45 Chenu and Cosentino, 2011).

46 Quantification of EPS in soils is a prerequisite for advancing the understanding
47 of beneficial roles of EPS. However, EPS extraction from soils is highly challenging
48 because commonly applied extraction methods typically co-extract high levels of
49 intracellular and extracellular contaminants. The source for intracellular
50 contamination is cell lysis and is usually quantified using microscopy and staining
51 methods to determine cell counts (Sheng et al., 2010). However, microscopy of soils
52 is highly challenging due to the abundance of opaque mineral surfaces and occlusion
53 within aggregates. DNA and ATP levels have been used as a proxy for cell lysis
54 (Takahashi et al., 2009). Extracellular DNA, however, is known to be an important
55 component of biofilms (Pietramellara et al., 2009; Dominiak et al., 2011), and thus
56 ATP may be more suitable for quantification of cell lysis. Major source for
57 extracellular contaminants in EPS extraction is non-biofilm soil organic matter that
58 has been successfully estimated by measuring humic acid equivalents (HAE) in
59 extracted EPS (Redmile-Gordon et al., 2014). Further, they found that the HAE
60 content of EPS extracts was determined primarily by the content of soil organic
61 matter but not by the extent of microbial biomass or EPS content as driven by
62 substrate additions. The HAE/EPS ratio in EPS extracts is therefore a useful indicator
63 of an extractant's 'specificity' for proteins and polysaccharides in soil microbial EPS.

64 Ultisols are widely distributed throughout the tropical and subtropical areas of
65 the world and occupy auout 8.7% of the global land (Eswaran, 1993). However, a
66 method to measure EPS in Ultisols, to our knowledge, has not yet been established.

Although Redmile-Gordon et al. (2014) demonstrated the applicability of cation exchange resin (CER) for extraction of EPS from a sandy soil, whether this method also suitable for extraction of EPS from Ultisols needed further study, because EPS bound by Fe^{3+} may be less readily extracted by CER owing to the trivalent forms exchange more difficult than divalent Mg^{2+} and/or Ca^{2+} (Wilén et al., 2003). Actually, Park and Novak (2007) demonstrated that CER was more selective for extraction of Mg^{2+} and Ca^{2+}-rich EPS, while sodium sulfide (SS) was more selective for extracting Fe-containing EPS from activated sludge. Here, CER, SS and several techniques which are usually used to extract EPS-like fractions from soils were investigated for extraction of EPS from Ultisols. We hypothesized that SS rather than the CER method may be more suitable for extraction of EPS from Ultisols.

Ultisols were sampled from 0-20 cm depth from a cedar forest located in He Shengqiao town, Xianning city, Hubei province, China (114°21′E, 30°1′N) and a paddy field of National Agro-Ecosystem Observation and Research Station in Yingtan city, Jiangxi province, China (116°55′E, 28°15′N).

In order to stimulate EPS production, soils were incubated with glycerol. More details on soil incubation and analysis were provided in Supplementary Materials. After incubation and removal of soluble microbial products from soils, five methods: hot dilute acid extractable polysaccharide (HDAEP), hot water extractable polysaccharide (HWEP), easily extractable glomalin (EEG), sodium sulfate (SS), and cation exchange resin (CER) method were used to extract EPS. The content of polysaccharides and proteins in extracts was quantified to evaluate EPS extraction

89 efficiency. HAE and ATP were measured to estimate the degree of extracellular and
90 intracellular contamination, respectively (see Supplementary Materials).

91 The comparison of the two polysaccharide extraction methods showed that
92 HDAEP method yielded 3 to 5 times higher levels of carbohydrates than HWEP
93 method ($p < 0.05$; Table 1). This was likely caused by hydrolysis of other organic
94 matter or plant tissues in HDAEP extraction, which overestimated polysaccharide
95 content (Redmile-Gordon et al., 2014). Total carbohydrate concentration (450-600
96 μg/g soil) in HWEP extract was higher than that reported in grassland soil (200-350
97 μg/g soil; Marchus et al., 2018). Protein content was very low (15 μg/g soil) in HWEP
98 extracts and not detectable in HDAEP extracts (Table 1), which was consistent with
99 the purpose of the methods optimized for extracting polysaccharides rather than
100 proteins. Moreover, due to the harsh extraction conditions (80 °C for 7 h, 0.125 M
101 H_2SO_4) the HDAEP method also caused severe extracellular contamination (Table 1)
102 and intracellular contamination (Fig. 1), indicating this method is unsuitable for
103 extraction of EPS from soils.

104 The EEG method yielded proteins (1.6-2.1 mg/g soil) (Table 1) that were
105 consistent with these found in the Atlantic Forest (Vasconcellos et al., 2016). This
106 method also extracted significant quantities of polysaccharide and non-proteinaceous
107 HAE (Table 1) and caused extensive cell-lysis (Fig.1), which was consistent with the
108 results of Redmile-Gordon et al. (2014). One would understandably interpret the
109 lowest HAE/protein ratio in EPS extracts (close to 1.0; Table 1) to mean that the EEG
110 extraction method was suitable for extracting non-specific protein from soils.

111 However, it should be noted that ‘protein’ as measured by the Bradford assay may be
112 incorrect: in part due to soil organic matter (SOM) derivatives quenching the
113 absorbance from protein, and partly due to direct ‘false positive’ measures from
114 nonspecific organic material (Redmile-Gordon et al., 2013).

115 CER has been widely used to extract EPS from active sludge, purely cultured
116 bacteria (Sheng et al., 2010) and sediments (Gerbersdorf et al., 2005) owing to its
117 high efficiency (Frolund et al., 1996), minimal contamination from the extractant per
118 se (Comte et al., 2006), and minimal cell lysis (Pellicer-Nàcher et al., 2013). Although
119 CER was less effective at extracting polysaccharides or proteins compared with some
120 of the other methods (Table 1), both extracellular contamination (Table 1) and
121 intracellular contamination (Fig. 1) were low, which is consistent with
122 Redmile-Gordon et al. (2014) who used CER to extract EPS from a Cambic Arenosol.
123 The EPS-polysaccharide (612-878 μg/g soil) was consistent with the estimate of EPS
124 contents (50-1400 μg/g soil; Chenu, 1995) and was higher than that in grassland (401
125 μg/g soil) and fallow soils (169 μg/g soil; Redmile-Gordon et al., 2014). This may be
126 due to higher amount of carbon addition (Redmile-Gordon et al., 2015) or different
127 soil physico-chemical properties (Rossi et al., 2012). By contrast, protein (163-182
128 μg/g soil) in our soil is comparable with that found in grassland (163 μg/g soil;
129 Redmile-Gordon et al., 2014) and a Chromic Luvisol (180-220 μg/g soil;
130 Redmile-Gordon et al., 2015), but higher than that in fallow soils (41μg/g soil;
131 Redmile-Gordon et al., 2014).

132 While CER was again found to be the most suitable method for conservative

133 extraction of EPS, it should be noted that the CER method may underestimate EPS
134 from Ultisols as EPS bound by Fe^{3+} may be more difficult to extract using CER
135 (Redmile-Gordon et al., 2014). Thus, other methods to extract iron-bound EPS are of
136 continued interest. The SS method indeed extracted 3-4 times higher polysaccharides
137 and 3-5 times higher proteins than CER ($p < 0.05$; Table 1). This seems consistent
138 with our hypothesis that SS rather than CER extracts more EPS, but the question
139 remains: what else does it extract?. The HAE in SS extracts was 15 times higher than
140 in CER extracts, thus resulting in significantly higher HAE/polysaccharides and
141 HAE/proteins ratios ($p < 0.05$; Table 1). In addition, the SS method decimated
142 microbial ATP compared with the CER method ($p < 0.05$; Fig. 1). Both of these
143 findings are likely due to the combination of heat and the strongly alkaline solutions
144 formed upon dissolution of sodium sulfide in water. While hydroxides enable very
145 thorough extraction of organic materials from soil, they also cause extensive cell lysis
146 (Liang et al., 2010) contaminate with non-EPS SOM, and confound the origins and
147 chemical properties of the extracted compounds (Schmidt et al., 2011). Where
148 possible, it is best to avoid confounding the true nature of these specific pools of SOM
149 (Lehmann and Kleber, 2015). Therefore, we cannot recommend the SS extraction and
150 instead maintain that extraction with CER offers the best balance between EPS-yield
151 and confidence of origin.

152 In conclusion, the HWEP and HDAEP methods were optimized for extraction of
153 polysaccharides rather than proteins. Although the EEG and SS methods extracted
154 more polysaccharides and proteins than CER, but these methods caused serious

intracellular and intercellular contamination. Thus, CER is currently the most appropriate method for extraction of EPS from Ultisols. More studies are required to evaluate the inclusivity of CER for extraction of EPS from soils.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (41877029), Royal Society-Newton Advanced Fellowship (NAF\R1\191017), the National Key Research Program of China (2016YFD0800206) and the Fundamental Research Funds for the Central Universities (2662017JC008).

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Table and Figure Legends

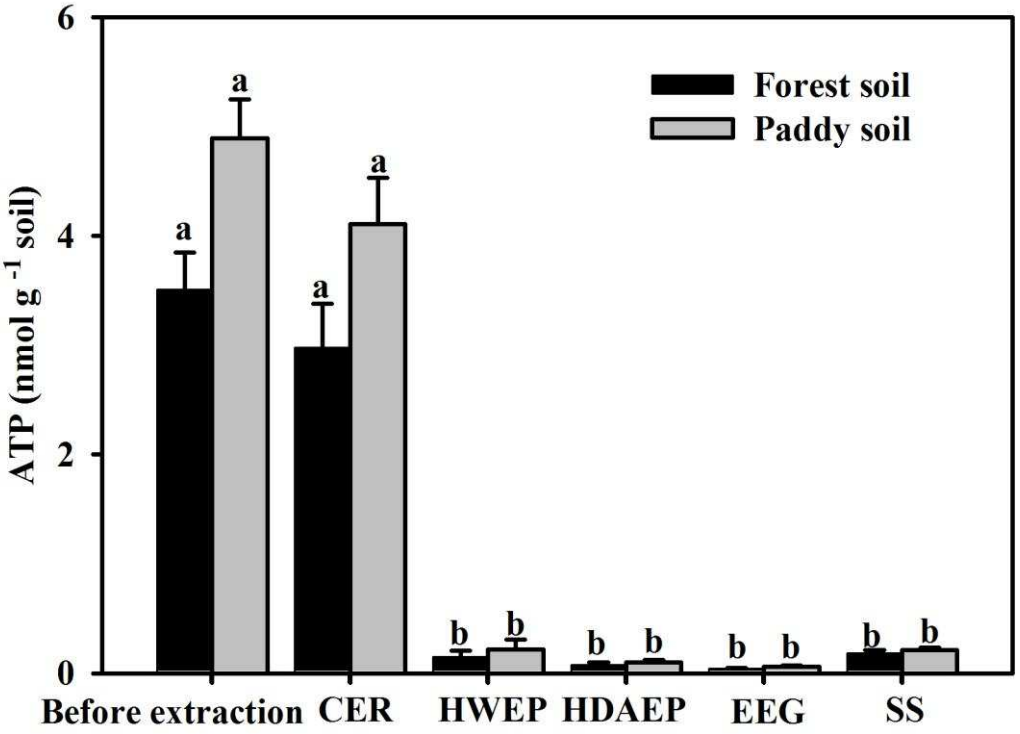
Table 1 EPS characteristics extracted by different methods. Data (means ± SE, n=3) annotated with different letters within a column indicate significant differences ($p < 0.05$) among the different extraction methods but in the same soil. N.D. indicates undetected (protein concentration below the detection limit). CER: cation exchange resin, HWEP: hot water extractable polysaccharide, HDAEP: hot dilute acid extractable polysaccharide, EEG: easily extractable glomalin (EEG), SS: sodium sulfate, HAE: humic-acid equivalent.

Fig. 1 Microbial biomass ATP content in different soils before and after extraction of EPS. Data (means ± SE, n = 3) annotated with different letters indicate significant differences ($p < 0.05$) in ATP content of the same soil before and after EPS extraction.

Table 1

Extract	Carbohydrate (μg glucose g^{-1} soil)		Protein (μg protein g^{-1} soil)		HAE (μg humic acid g^{-1} soil)		HAE/Carbohydrate		HAE/Protein	
	Forest soil	Paddy soil	Forest soil	Paddy soil	Forest soil	Paddy soil	Forest soil	Paddy soil	Forest soil	Paddy soil
CER	612±50d	878±69D	184±17c	163±13C	440±19d	388±9D	0.72±0.07d	0.44±0.03C	2.41±0.29c	2.38±0.20C
HWEP	447±20e	598±23E	15±3d	14±3D	44±4e	150±10E	0.09±0.01e	0.25±0.02D	2.71±0.43b	10.71±2.13A
HDAEP	1314±152c	2970±80A	N.D.	N.D.	3169±92b	1142±52C	2.44±0.31b	0.38±0.02C		
EEG	1620±130b	2103±191C	1438±62a	2450±163A	1851±61c	1599±43B	1.15±0.08c	0.76±0.06B	1.29±0.10d	0.66±0.06D
SS	2222±111a	2417±185B	493±27b	818±42B	6236±343a	6216±276A	2.80±0.22a	2.58±0.21A	12.64±0.50a	7.62±0.52B

Fig.1


6
4
2
0
ATP (nmol g-1 soil)
Forest soil
Paddy soil
a
a
a
a
b
b
b
b
b
b
b
b
Before extraction
CER
HWEP
HDAEP
EEG
SS